Peat source and its impact on the flavour of Scotch whisky

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Abstract

Peat is used as a source of flavour compounds in Scotch malt whisky production when it is burned during malt kilning. The aim of this project was to establish whether peats from different locations in Scotland are chemically distinct and could consequently impart variations in flavour to malt whisky. Peat samples from four geographical locations (Islay, Orkney, St Fergus and Tomintoul) were distinguished by Fourier Transform Infrared (FT-IR) spectroscopy. Analysing peat samples using Curie point pyrolysis in combination with Gas Chromatography-Mass Spectrometry (Py-GC-MS) showed that peats from Islay and St Fergus were rich in lignin derivatives while those from Orkney and Tomintoul had higher levels of carbohydrate derivatives. Also, Islay and Orkney peats were rich in nitrogen-containing heterocycles and aromatic hydrocarbons respectively. A laboratory-scale peating process was developed and malt was prepared using peat from the four locations. This malt was mashed and fermented and the wash distilled on a laboratory-scale. Analysis by GC-MS showed that some peat-derived compounds such as lignin-derived syringols were not transferred to the spirit. Nevertheless, principal components analysis of the GC-MS data revealed that the spirits grouped into a similar pattern as that derived from the peat analyses. Assessment of the spirits by sensory panel revealed significant flavour differences; in particular spirits prepared with Islay peats were distinguished by burnt and smoky aromas. These findings indicate that if distillers change the source of their peat, either through choice or by necessity, they must take into account the possible influence that this could have on the flavour character of their product.

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1.1.1 The history and development of malt whisky

The art of distillation is thought to have first been attempted in Asia as long ago as 800 BC and to have found its way to Europe via Egypt [1]. In the British Isles it is unclear as to whether the practise originated in Wales, Ireland or Scotland. The first recorded mention of distilling in Scotland was in 1494 in the Exchequer Rolls: 'And by payment made to Brother John Cor by precept of the comptroller, as he asserts, by the King's command, to make aquavite within the period of the account, 8 bolls of malt' [2]. Aqua vitae (the water of life) is the generic Latin name given to early distilled alcoholic beverages irrespective of their basic raw material. The Gaelic translation of aqua vitae is 'uisge beatha', the corruption of which gave 'uisge' then 'usky' and finally whisky which became an official name in 1755 when Dr Samuel Johnson entered the word in his dictionary [1].

In Scotland, distillation was originally the domain of the abbeys and monasteries. During the sixteenth century, the dissolution of the monasteries resulted in the spread of distilling knowledge from the monks to others. The resulting widespread production of whisky necessitated the Act of the Scottish Parliament of 1579 which allowed whisky production by lords and 'gentlemen' only [1]. This was an attempt to decrease the detrimental effect distillation was having on grain supplies. After the Union of the Parliaments in 1707, English revenue staff crossed the border to begin their lengthy attempts to bring whisky production under control. It was realised that significant revenue could be collected from the ever growing distilleries of Scotland, and by 1756 duty paid on spirit had increased to eight times that of 1708 [3]. A trend of stricter regulation and increasing taxation characterised subsequent years as well as short periods of prohibition, generally provoked

by disastrous grain harvests. Illicit distilling flourished, the smugglers seeing no good reason for paying for the privilege of making their native drink [1].

By 1823, the excise laws were in such a hopeless state of confusion that no two distilleries were taxed at the same rate and so a new Excise Act which sought to temper previous regulations with more manageable and less punitive legislation was passed. Distillers could now buy a license for £10 and with reduced duty, many unlawful stills now emerged as the registered distilleries known today [4]. Following the Act of 1823, there was a dramatic rise in legal whisky production (within two years production almost tripled) [1]. During this time, a differentiation was made between grain and malt distilling with Highland whisky becoming exclusively single malt while Lowland grain distilleries used only a small amount of malt to saccharify cooked unmalted grain.

The expansion of the whisky industry was driven by several technical developments. Grain whisky production increased due to the development of continuous stills. One of the reasons whisky dominates the world market, rather than Cognac for example, is that large amounts can be made using continuous stills. The use of casks to store whisky and allow it to mature gave a product which was more appreciated by the consumer. The blending of both grain and malt whiskies allowed the production of consistently high quality products in large quantities.

The consequence of these new products based upon spirit from continuous stills resulted in dispute between the malt and grain distillers, and the blended whisky producers whose opinion of what should be legally sold as Scotch whisky conflicted. In November 1905 Islington Borough Council raised a trial case asking the question, "What is Whisky?" the deliberation of which resulted in the magistrate ruling in favour of malt whisky. Inevitably, the pronouncement was appealed by the grain manufacturers and blenders, however no resolution was reached as the jury were equally split. Government intervention was desired and following further arbitration it was decreed that a formal definition of whisky would be decided by a Royal Commission [1]. Ironically, the resulting legislation allowed for the sale of grain, malt and blended products as Scotch

whisky. This was crucial, if the legislation had limited Scotch whisky to malt alone, the industry would have remained small. Today, this legislation exists in the Scotch Whisky Act 1988 and the Scotch Whisky Order 1990 which states that Scotch whisky is:

Whisky -

(a) which has been produced at a distillery in Scotland from water and malted barley (to which only whole grains of other cereals may be added) all of which have been –

(i) processed at that distillery into a mash;

(ii) converted to a fermentable substrate only by endogenous enzyme systems; and

(iii) fermented only by the addition of yeast;

(b) which has been distilled at an alcoholic strength by volume of less than 94.8 per cent so that the distillate has an aroma and taste derived from the raw materials used in, and the method of, its production;

(c) which has been matured in an excise warehouse in Scotland in oak casks of a capacity not exceeding 700 litres, the period of that maturation being not less than 3 years;

(d) which retains the colour, aroma and taste derived from the raw materials used in, and the method of, its production and maturation; and

(e) to which no substrate other than water and spirit caramel has been added [5].

As a result of the legal restrictions on the starting material, geographical location and time frame for maturation, malt whisky production in Scotland is now a well established process with little scope for variation.

1.1.2 Scotch malt whisky production process

The following are descriptions of the major processes carried out during the production of Scotch malt whisky. These descriptions have been taken from the Alcohol Text Book unless otherwise stated [6].

Malting

The cereal used in the production of Scotch malt whisky is barley. This in turn is converted into malt, a substrate suitable for alcohol production, by way of the malting process. This process can be divided into three stages: steeping, germination and kilning.

Steeping- The steeping of barley involves the uptake of water in order to initiate uniform germination and to hydrate the grain endosperm to a level that is suitable for modification.

Germination- Once the required moisture level is achieved the barley grains are allowed to germinate for a period of time. For malt spirit production, the purpose of germination is to maximise the fermentable extract by promoting both endosperm modification and the development of amylolytic enzymes. The amylolytic enzymes are responsible for the production of fermentable sugars from starch. In the manufacture of Scotch malt whisky, where only malted barley is used with no other grains included, care must be taken when the grain is sprouting during the malting process to ensure that only a limited amount of amylolytic enzyme activity is produced. This is because enzymes are produced at the expense of the fermentable extract in the grain. Therefore, progress and uniformity of germination are controlled by managing optimum moisture levels within the barley, supplying air, removing carbon dioxide and excess respiratory heat and mechanically turning the grain to prevent matting of rootlets.

Kilning- The purpose of kilning freshly produced or green malt is to stop biological activity when the required enzyme levels and degree of modification have been reached, and to produce a dry storable product that can be milled to the grist required for mashing.

To this end, the green malt is dried using a low temperature which allows enzymic activity to be retained. Undesired flavour components such as sulphur compounds, (especially sulphides) are driven off [7], while other desired ones appear- either from existing precursor chemical compounds [8] or from extraneous sources such as peat smoke [9]. The processes taking place during kilning are summarised in Figure 1.1. Moisture is removed from the malt in an unrestricted manner until malt moisture of about 20–25% is reached- the free drying stage. Free drying is terminated by the break, which represents the drying front progressing through the malt bed in the direction of the air stream until it breaks at the surface. Water remaining in the malt is released less readily and as a consequence, temperature of the malt starts to rise- the diffusion stage. This stage continues until a moisture content of approximately 10–12% is reached. At this point, most of the water is bound and further moisture is only released by increasing the temperature of the drying stream. By doing this, the moisture of the malt may be further reduced to a value between 4–6%. During free drying, modification and enzyme synthesis continue. After the break, enzyme activity declines and some enzymes are destroyed, the latter process accelerating during the final stages of kilning [10].

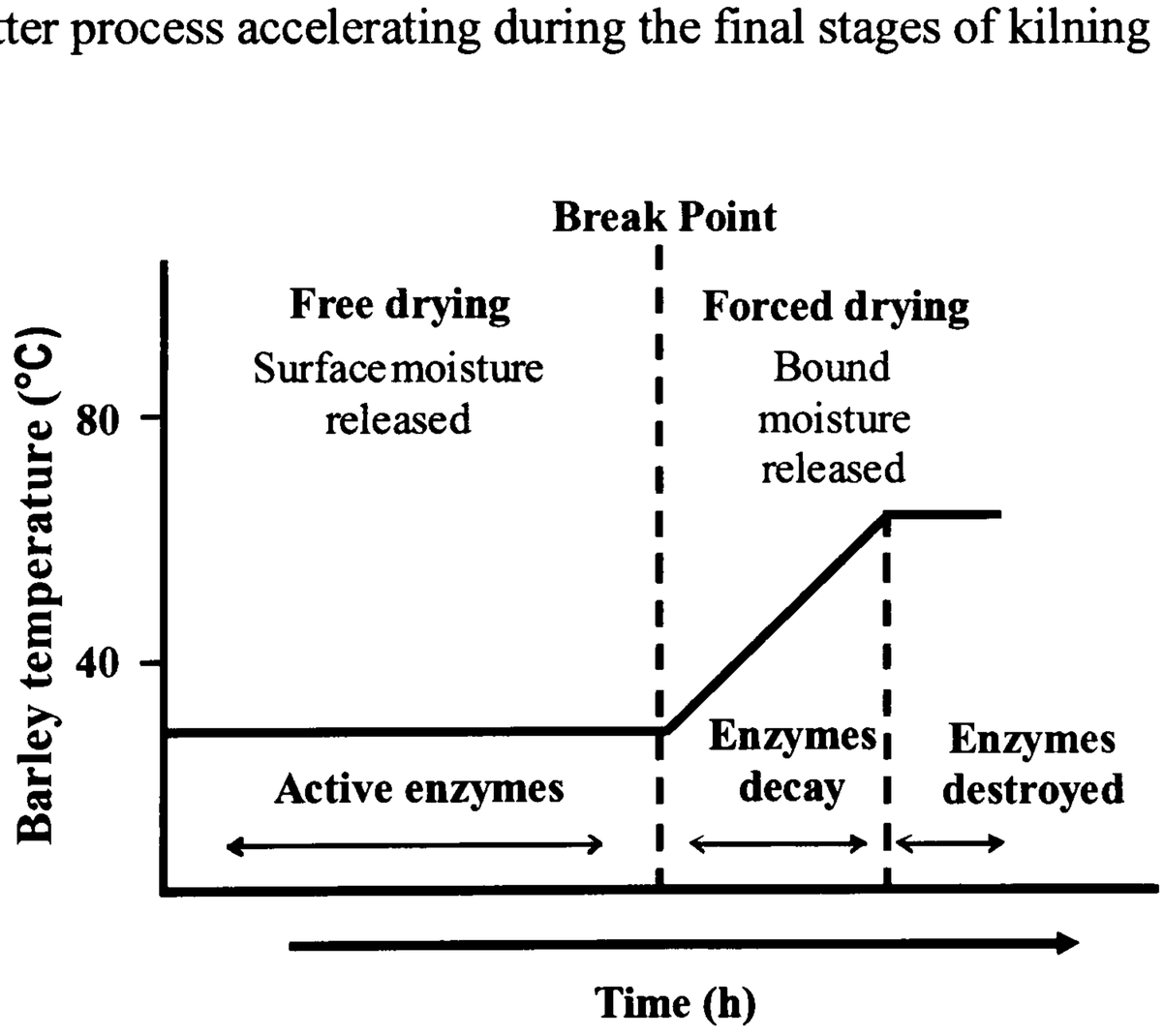


Figure 1.1. Processes taking place during kilning of green malt [11].

In some cases during kilning, peat is burned, avoiding flaming, to produce a smoke called peat reek. Careful control of combustion temperatures is necessary to achieve the desired

degree of peating. Adsorption of peat smoke on to malt is not a simple operation and is governed by both the intensity of the smoke and the rate of malt drying [12]. Peat smoke will not readily adsorb on to malt when there is a surface water film, i.e. during free drying, and adsorbs only slowly when the surface is very dry. Greatest adsorption occurs when malt is hand dry. Peat smoke must therefore be applied very intensely during the early period of kilning right up to and beyond the 'break point'.

Milling

Malt is milled to produce a grist which can be mixed with water during mashing to allow efficient gelatinisation, enzymolysis and dissolution.

Mashing

After milling, the resultant grist is mashed in a mash tun. The main biochemical changes that take place during mashing are the enzyme catalysed hydrolysis of starch, protein and other biopolymers in the grist to produce water soluble low molecular weight compounds that form a fermentable substrate (or wort). The major starch-liquefying and saccharifying enzymes are α- and β-amylases, while the limit dextrins formed by action of amylases on amylopectin are further hydrolysed by limit dextrinases.

Fermentation

The wort, or clear mash, leaving the mash tun is cooled and fed into a vessel (a "washback") where it is mixed with yeast. Fermentation is conducted with strains of the yeast *Saccharomyces cerevisiae* that are usually specially propagated for the purpose, although this may be supplemented with small quantities of surplus brewer's yeast. During fermentation, mash sugars are converted to ethanol and carbon dioxide. In addition, minor amounts of other organic compounds are produced which contribute to the organoleptic qualities of the final distilled product.

Distillation

The key objectives of distillation are the concentration of alcohol and selective separation of the volatile compounds (particularly the flavour-producing congeners) from the non-

volatile compounds. Additional flavour compounds are produced as a result of chemical reactions that take place in the still. Pot stills used for malt distillation are traditionally constructed of copper. The reason for this adherence to copper is more than tradition. Fermented wash contains relatively high levels of volatile sulphur-containing compounds, which would be undesirable in the final product. Interaction with copper limits their concentration in the spirit.

Scotch malt whisky production normally involves a two stage pot still distillation. The only exception to this is Auchenthoshan Distillery, which has a third distillation step. The fermented wash is fed directly to the first still, known as the wash still. This wash distillation effects a three-fold concentration of alcohol. The residue in the wash still, known as pot ale, is either discharged to waste or evaporated to produce animal feed. From the wash still, the distillate is redistilled in the second still, referred to as the low wines or spirit still. This second distillation is more selective than the wash distillation. The first fraction, which contains low boiling point compounds, is rejected as foreshot heads. At a stage determined by continued hydrometric monitoring, which usually occurs when the distillate has an alcohol content of approximately 70–73%, the distillate is switched from the foreshots tank to the whisky receiver tank. This switch has traditionally been made at the discretion of the distiller; and he has been aided by the disappearance of a bluish tinge when water is added to the distillate. The monitoring process takes place in a spirit safe secured with a Government Excise lock. Collection of the distillate is terminated when the alcohol content has fallen to a specified value, although distillation of the feints or tails is continued until all of the alcohol has been removed from the low wines. The whisky distilled over in the middle fraction, or spirit cut, has an alcohol content of 63–70%. The foreshots and feints are recycled, while the residue remaining in the spirit still, known as spent lees, is either run to waste or evaporated to manufacture animal feed.

Maturation

Freshly distilled whisky of any type is very different from the spirit that is later bottled, either singly or blended. The transformation is brought about by storing the whisky in

oak casks for periods of time that depend on traditional practice and legal requirements. In Scotland, where maturation takes place in cool, unheated, but humid warehouses, the alcoholic strength decreases [13]. During maturation, changes occur in the chemical composition of the whisky. These changes are attributable to extraction of wood constituents, oxidation of components present in the original whisky as well as those extracted from the wood, reactions between components in the whisky and removal and oxidation of highly volatile sulphur components by the carbon char on the inner surface of the cask.

1.1.3 Scotch malt whisky flavour

The Scotch Whisky Flavour Wheel

The flavour of Scotch whisky is complex. This complexity is illustrated by the Scotch Whisky Flavour Wheel in Fig. 1.2 which shows the large number of descriptors that can be applied to whisky aroma. This wheel is organised into a hierarchy of three tiers:

- Primary (centre of the wheel): describes the production origin or the general nature of the aroma.
- Secondary: specific sensory descriptors
- Tertiary: highly specific sensory descriptors of technical importance

The aromas on the right of the flavour wheel are generally considered to arise from normal production and are suitable for promotional and marketing purposes [14]. Amongst these are the peat-derived or peaty aromas which can be further subdivided into burnt, smoky and medicinal. These peaty aromas are known to be key quality attributes for certain Scotch whiskies [15].

Conversely, the oily, sour, sulphury and stale aromas located on the left of the flavour wheel are often considered as off notes and these descriptors tend to be used for technical rather than promotional functions [14].

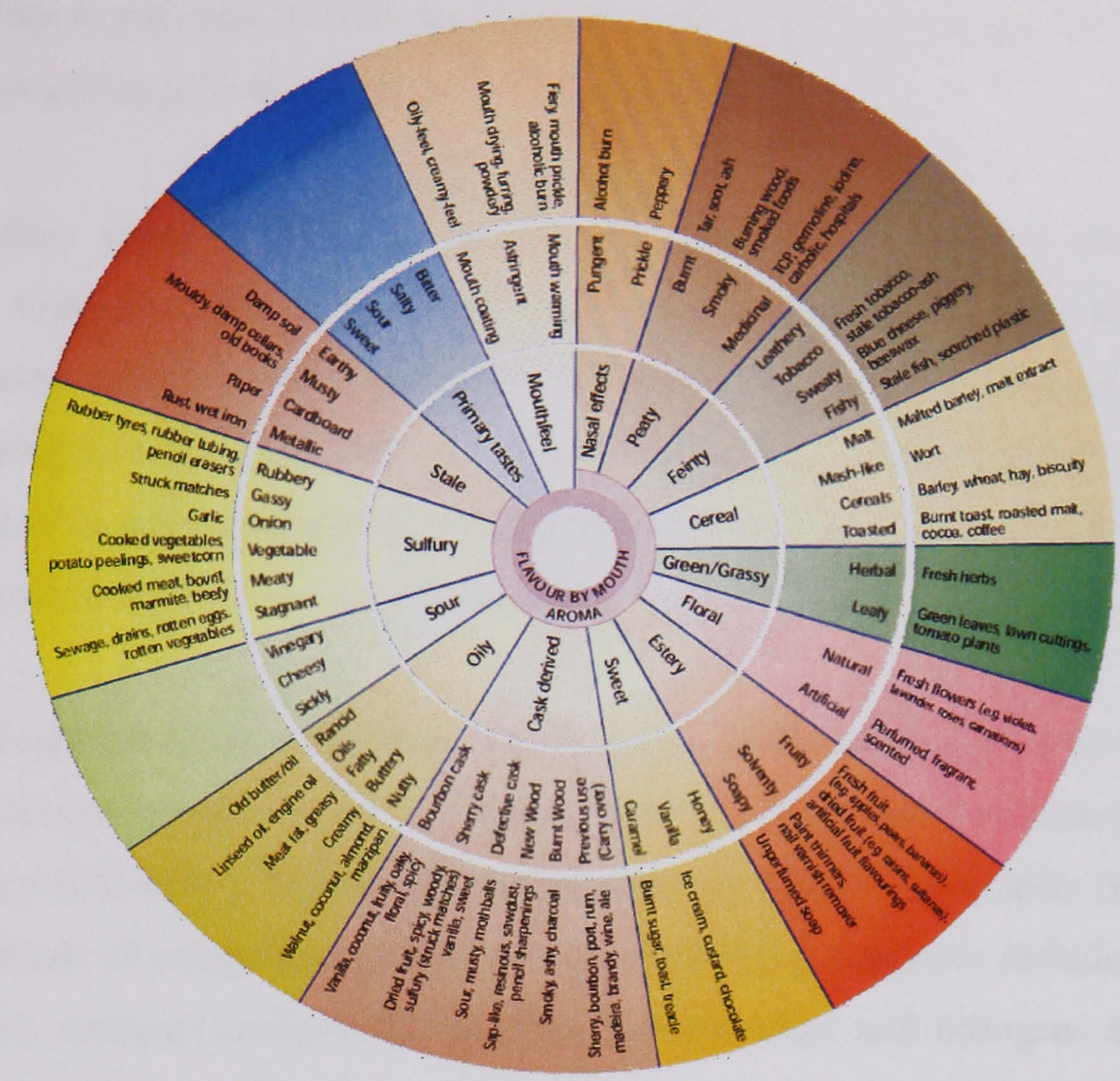


Figure 1.2. The Scotch Whisky Flavour Wheel (produced by the Scotch Whisky Research Institute [16].

Compounds contributing to Scotch whisky flavour

Although whisky has a history going back hundreds of years, little was known about its composition until the late nineteenth century. In 1891, when describing methods for the detection of alcohols, aldehydes, esters, amines and furfural, Allan and Chataway made two very important points [17]:

> *The secondary constituents of spirits are by no means to be regarded in the light of impurities, as they have been wrongfully called and considered by some. They are the associated bodies which give the alcohol its special and valued characters…*

> *The secondary or bye products of spirits are naturally most abundant in those spirituous liquids manufactured in apparatus where no, or but little, fractionation*

> *occurs. This is the case with the spirit distilled in Scotland distilled in pot stills and made wholly from fermented malt...*

The secondary constituents to which Allan and Chataway refer are known as the congeners, these are trace components found in mature whisky as a result of the raw materials used and the various processing steps involved in whisky production. After bottling, congeners make up only about 0.1% of the final composition of whisky [18] (water makes up approximately 59.9% and ethyl alcohol 40%) yet it is these congeners which give whiskies their sensory characteristics.

The analytical knowledge of whiskies has expanded rapidly starting with the introduction of gas chromatography (GC) in the 1960s [19,20], gas chromatography-mass spectrometry (GC-MS) [21] and high performance liquid chromatography (HPLC) [22]. Many hundreds of congeners have now been identified in whiskies including alcohols, acids, esters, carbonyl compounds, phenols, hydrocarbons, and nitrogen- and sulphur-containing compounds [23]. Additionally, gas chromatography-olfactometry (GC-O), a technique first proposed in 1964 [24], has been used to identify compounds contributing to whisky aroma [25,26].

It must be noted that while analytical methods can be used to identify and quantify aroma-active compounds in whisky, it is often the case that the levels of aroma compounds in a mixture such as whisky can not be directly related to sensory perception [16]. For example, combinations of some compounds can have synergistic [27] or masking effects [28]. Additionally, some compounds with no aroma activity of their own can exert an effect on the perception of others. This was found to be the case with long chain fatty acid ethyl esters in whisky where these compounds trapped other aroma-active compounds in the liquid phase of the whisky preventing them from contributing to the aroma [29].

1.2 Peat

1.2.1 Peatlands

There is no internationally accepted definition of peat. Shotyk defined peat as "light brown to black organic sediment formed under waterlogged conditions from the partial decomposition of mosses and other bryophytes, grasses, shrubs or trees" [30]. The northern peatland ecosystems cover 2.3% of the Earth's land surface and they have sequestered about 25% of the carbon in soils worldwide [31]. All peatlands comprise two layers: an acrotelm and a catotelm, the separation of which is defined by the water table [32]. The acrotelm roughly approximates to the upper aerobic layer of between 10 to 50 cm and the catotelm represents the lower, waterlogged anaerobic layer. Peat forms slowly, taking approximately 3000–4000 years to accumulate a depth of 1 m of peat [33]. Peat decomposes slowly because of limited oxygen availability for microbial respiration in the waterlogged portion of the peat profile as well as the inherent low decomposability of plant litter and cool temperatures of peat [31].

There are two main types of peatland system: ombrotrophic and rheotrophic peatlands. This classification is based on the origin of the peatland water, ombrotrophic peatlands are supplied exclusively by precipitation while rheotrophic peatlands are additionally supplied by ground water from the surrounding soil [34]. Ombrotrophic peatlands tend therefore to be lower in inorganic mineral components (ash content of 3% compared with 3 to 14% for rheotrophic). Ombrotrophic peatlands are also more acidic (pH 3 to 5 compared with pH 4 to 7.5 for rheotrophic) [33].

Scottish peatlands

It is estimated that the total area of peat in Scotland, with a minimum thickness of two feet, is of the order of 2 million acres [35]. In Scotland, peatlands can broadly be divided into climatic or zonal deposits and local or azonal deposits. As described by Fraser, the following is an overview of these types of peatland [35].

Climatic (zonal) deposits

This type of deposit is ombrotrophic and occurs in the form of blanket bog and hill bog. Due to its ombrotrophic nature, blanket peat requires an abundant rainfall fairly evenly distributed throughout the year so that the surface of the soil is more or less continuously saturated and a temperate climate with cool summers and mild winters. These conditions are typical of a cold, temperate oceanic climate and in Scotland are most fully satisfied in the west and north of the mainland and on the islands off the western and northern coasts. Generally speaking, blanket bogs are comparatively thin showing little variation in composition from top to bottom. As a rule, the profile starts below as a layer of buried humus derived from the previous vegetation, but very soon changes to the *Sphagnum* or similar peat of which the rest of the deposit is formed. Usually no well defined zones can be observed. The relative shallowness of blanket peat is due mainly to the fact that the surface of the peat is soon raised above the range of mineral nutrients. The uniformity of composition is an expression of the general uniformity of the climatic conditions prevailing throughout the period of accumulation. Vegetation found on a typical blanket bog consists of varying amounts of deer grass, heather, cotton grass, bog asphodel, purple moor grass and other moorland plants (species of sedge and rush).

Hill peat is, like blanket peat, climatic in origin and regional in distribution. It is formed as a result of the wetter and more extreme climate of moderate elevations in mountainous country. As such, hill peat is a characteristic and widespread type of deposit in Scotland. Hill peat differs from blanket peat climatically as they are subject to increased atmospheric precipitation; they undergo marked seasonal variations in temperature as well as periodic exposure to desiccating winds. Hill peats, due to their surface configuration combined with exposure to sun and strong wind, experience periodic surface drought. Hill peat normally occurs in the altitudinal range from 1000 to 2000 feet but this range may be reduced in more exposed areas. The profile of hill peat, like that of blanket peat tends to be composed of the same types of plant remains from surface down to basal humus. Hill peat, however, tends to be darker in appearance, denser and more fibrous than typical blanket peat, at least near the surface. These differences are due to hill peat being more liable to extremes of temperature and its subjection to periodic

surface drought. In terms of vegetation, hill peats tend to be more abundant in heathers and cotton grass than blanket peats.

Local (azonal) peat deposits

The characteristic feature of local deposits, such as basin or valley peats, is the fact that they begin to form under the influence of ground water and as such are rheotrophic in nature. Whilst the influence of ground water persists, the deposit is said to be in the low moor stage. The term low moor can be used to cover a whole series of aquatic peat types varying from the English fen type which is high in nutrients through the similar reed grass lake peats of Eastern Scotland which are intermediate in character to the *Sphagnum*-sedge and *Sphagnum* aquatic peats of the West of Scotland which are low in nutrients and acidic. When, however, continued growth raises the surface of the deposit above the influence of the ground water, so that the vegetation and any subsequent increase in thickness of the peat (as in blanket bog) depends essentially on rainfall, it is said to have reached the raised bog stage. Broadly speaking, the local peat deposits in Scotland have passed beyond the low moor stage. When the peat starts to form above the low moor stage, the usual consequence is for formation to continue with definite and often marked changes in vegetational character. In the high nutrient types the rank, lush vegetation is replaced at first by slower growing species: reed grasses, for example, become less common and are replaced by *Sphagnum* and sedges, and by cotton grasses. Also, the earlier more robust species of *Sphagnum* are replaced by short, fine leaved forms and ultimately the vegetation consists of plants such as heathers and cotton grasses. In the more acid, low nutrient basin peats of the west and north of Scotland the *Sphagnum* or *Sphagnum*-sedge vegetation which is typical of the later stages of the low moor stage is displaced by finer leaved and slower growing species of *Sphagnum* as well as such plants as smaller sedges, heather and cotton grass. In raised bogs there may also occur narrow zones of peat which represent periods when plant growth rate and decomposition rate were very similar. These zones are thought to have been formed during dry periods when heathers and even pine trees were allowed to grow. On return of wetter conditions, *Sphagnum* and cotton grass swamped the pine-heather humus.

1.2.2 Peat chemical analysis

Peats from different sources have previously been chemically differentiated using Curie-point pyrolysis-mass spectrometry (Py-MS) [36,37]. While this approach provides information on the relative contributions of chemical species, it is destructive and there are long term reproducibility issues [38]. By contrast, Fourier Transform Infrared Spectroscopy (FT-IR) is a vibrational spectroscopic technique which measures the absorbance of infrared light by functional groups in molecules [39]. FT-IR is rapid, automated, non-destructive and is not biased to any particular group of chemicals and so generates 'holistic' fingerprints of the biological samples under investigation [40,41]. FT-IR spectroscopy has been used previously in the characterisation of peat from different depths in a profile [42,43] and distinct vegetation zones in and bordering a Scots pine woodland [44]. With 882 data points for each spectrum collected, using a high throughput screening technique such as FT-IR produces large data sets. The analysis of such complex data requires the application of multivariate statistical methods such as principal components analysis (PCA), discriminant function analysis (DFA) and hierarchical cluster analysis (HCA). These methods allow the detection of any clustering in the data and thus samples with similar chemical properties can be identified.

Though FT-IR could be used as a convenient screening method, it does not provide information on the specific chemical compounds responsible for any clustering in data. Analytical pyrolysis in combination with gas chromatography-mass spectrometry (Py-GC-MS) has previously proved very useful for the chemical characterisation of recalcitrant macromolecules such as those found in peat [45-47]. One popular pyrolysis technique for the analysis of peat is Curie point pyrolysis where the pyrolysis sample carrier is a ferromagnetic material which heats up in a very short time inside a magnetic field to a well defined Curie point temperature. The products of pyrolysis can then be identified using GC-MS. As the peat sample is thermally degraded during this analysis, this method reflects the process undergone during the incomplete combustion of peat in a kiln.

1.2.3 Chemistry of peat

General chemical composition of peat

The chemical composition of peat is derived from a combination of plants and microorganisms, the soil water quality and the secondary substances produced during the decomposition process. An overview of the chemical composition of a peat is given in Fig. 1.3 (taken from [48]). Simple sugars and amino acids and other water soluble components of the living tissues are present in low concentrations in peat or may even be entirely absent. More persistent, less reactive structural elements, such as lignin, contribute to the solid structure.

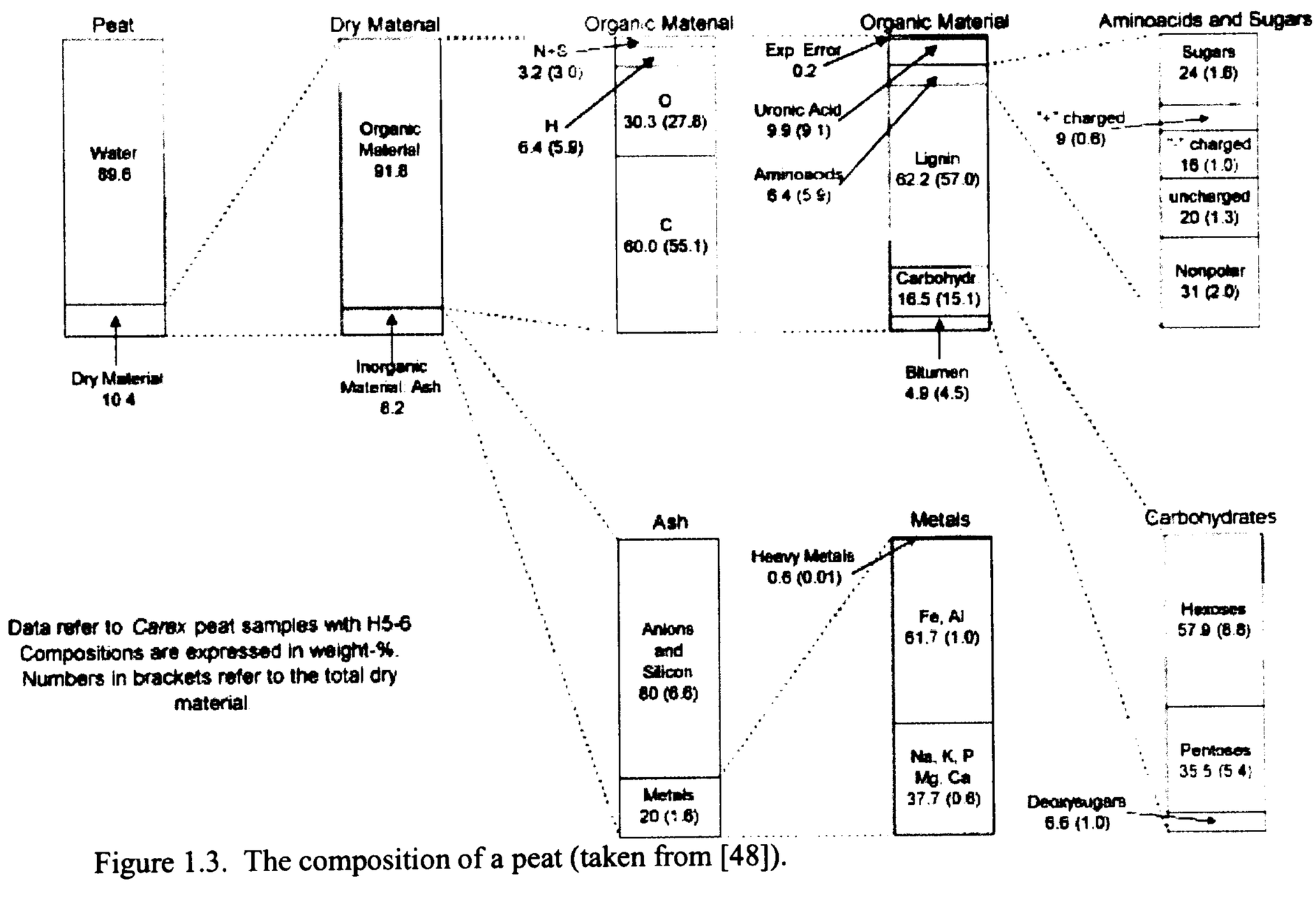


Figure 1.3. The composition of a peat (taken from [48]).

Lignin

In a previous study using Py-GC-MS to analyse the chemical composition of peat [36], the most significant chromatographic peaks identified were due to phenolic compounds related to lignin. Lignin is the second most abundant polymeric organic substance in the world [49]. Lignin is characteristic of the tissues of higher plants such as gymnosperms

and angiosperms, where it typically occurs in the vascular tissues [49]. The monomeric units from which lignin is biosynthesised have characteristic substitution patterns around the benzene ring. Lignin precursors are believed to be *p*-coumaryl, coniferyl and sinapyl alcohols- the monolignols (Fig. 1.4) [50].

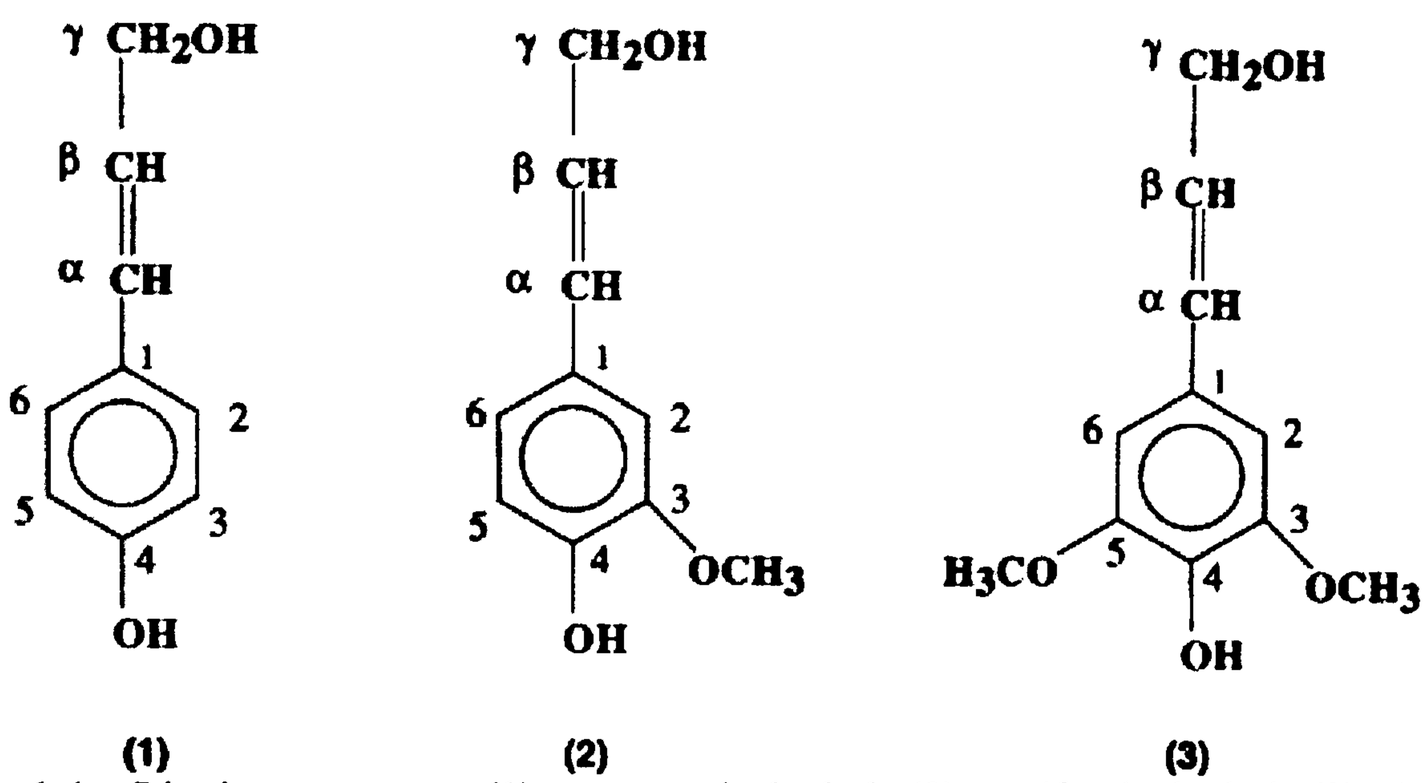


Figure 1.4. Lignin precursors. (1): coumaryl alcohol, (2) coniferyl alcohol, (3) sinapyl alcohol. Taken from [50].

The relative proportions of these monomers in lignin vary between different vascular plant species [51]. Gymnosperm (non-flowering plant) lignin is a polymer of coniferyl alcohol. Most angiosperm (flowering plant) lignin is a mixed polymer of coniferyl and sinapyl alcohols. Grass lignin though, is composed of a mixed polymer of coniferyl, sinapyl and *p*-coumaryl alcohols. Additionally in grass lignin, aromatic or cinnamic acids are esterified to the Cγ-hydroxyl group of the side chains in the lignin polymer [51].

Sphagnum mosses are examples of non-vascular plants which are characterised by a type of polyphenolic network which is different from the true lignin present in vascular plants [52]. *Sphagnum*-derived peat gives a high yield of *p*-hydroxyl phenolics relative to syringyl and guaiacyl phenols resulting from a lack of mono- and dimethoxyl substituted phenyl propene monomers in its polyphenolic network. This serves as an organochemical fingerprint of *Sphagnum* [53].

Cellulose

The major component of wood, cellulose, makes up approximately one half of both softwoods and hardwoods. Cellulose can be characterised as a linear high-molecular-weight polymer built up exclusively of β-D-glucose (anhydroglucopyranose) units [49]. Cellulose acts as the main structural component of plant cell walls.

Hemicellulose

Hemicelluloses are found in close association with cellulose in the cell wall. Hemicelluloses differ from cellulose by inclusion of various sugar units, by much shorter molecular chains, and by a branching of the chain molecules [49]. Five neutral sugars: glucose, mannose and galactose (the hexoses) and xylose and arabinose (the pentoses) are the main constituents of hemicelluloses. Some hemicelluloses additionally contain uronic acids. Traditionally, hemicelluloses are classified into hexosans, pentosans and polyuronides. This is a rough classification which does not take into consideration that the sugar units from different groups are mixed in most hemicelluloses. The classification according to the main components of the respective hemicellulose has proved useful for many years. In this system the hemicelluloses are classified as xylans, mannans, galactans etc. [49].

1.2.4 Factors affecting peat chemistry

Peat vegetation

The type of vegetation found in a peat deposit is dependent on, as mentioned above, the particular climatic and environmental conditions of the deposit. The chemical composition of the vegetation can vary according to, for example, its lignin composition. The initial composition of vegetation input to a peat deposit thus plays an important role in the chemistry of the peat.

Peat decomposition

The degree of decomposition or humification can be seen as the degree to which the fibrous content of bog plants have decomposed into amorphous (non-fibrous) solids [33].

In 1926 von Post developed a humosity grade in order to describe the degree of decomposition of peat [54]. This is a basic method where a small amount of peat is crushed in the hand and the colour of the water running out and the nature of the residue are used to assess the degree of decomposition determined in values from H1 (completely unhumified) to H10 (completely humified). Various other methods including microscopic, gravimetric, chemical extraction, centrifugal and optical methods have been adopted but are not very reproducible [55]. More direct measurements such as ^{13}C-nuclear magnetic resonance spectroscopy (^{13}C-NMR) [56,57] and Py-MS [37,58,59] have proven to be more useful for the characterisation of peat decomposition products.

The degree of decomposition can have an important effect on peat chemistry: a study of three different peatlands showed that chemical composition and indices of decomposition differed markedly between sites despite a general similarity in botanical composition [31]. The decomposition of peat is usually considered to parallel the size of peat particles; more decomposed, or sapric, peat gives finer particle size fractions than less decomposed or fibric peats [56]. Generally, as particle size decreases, the polysaccharide character of peat is greatly diminished with less hexose, pentose and extensin character and lignin biodegradation products dominate [60].

The most active organisms in the aerobic acrotelm layer of peat are fungi which can degrade both polysaccharides and lignin [61]. Unlike cellulose and other carbohydrates, lignin is resistant to degradation by most microorganisms. The principal lignin-degraders are the "white rot" fungi [62]. Lignin decomposition is, for the most part therefore, an aerobic process utilising fungal oxidase enzymes [31]. Lignin chemistry may be particularly sensitive to different environmental conditions in the acrotelm which will affect the fungal population. Infra red (IR) and chemical analysis of decayed lignins have revealed three principal changes: (1) oxidation of side chains, leaving aromatic, particularly vanillic, acid residues, (2) oxidation of the α-carbon in the propanoid side chains, and (3) the cleavage of aromatic rings still attached to the polymer [63].

As diffusion is unable to compensate for the rapid respiration of degrading organisms, oxygen abruptly disappears at the groundwater/air boundary [64]. Therefore, in the underlying anaerobic catotelm layer, fermentative/methanogenic bacteria utilise polysaccharides preferentially over lignin [61,65,66].

Several factors can have an effect on the rate of decomposition. When creating a model for peat decomposition, Frolking et al. [67] identified three mechanisms responsible for slowing decomposition rate with depth: reduction in temperature, increasingly anaerobic conditions and also as litter decomposes, it becomes more resistant to further decomposition. These factors act by having an impact on the populations of decomposing organisms found in peat. In a New Jersey rheotrophic peat, bacterial population diminishes rapidly from about 6×10^6 g^{-1} of moist peat at surface to 2×10^4 at 90 cm, but increases at still greater depth to the range $1–5 \times 10^5$ [68]. These results reflect a decrease in aerobic bacteria and an increase in anaerobic bacteria with depth. In a Maine ombrotrophic *Sphagnum* peat the surface bacterial populations were low in the top 30 cm of peat, ranging from $1–2.5 \times 10^5$ g^{-1}, but increased thereafter, reaching a maximum of 3.5×10^6 at a depth of 45–60 cm. Below that, populations were slightly lower but always much greater than at the surface. Predominant bacterial forms throughout the ombrotrophic peat were of the aerobic or facultative aerobic types. Waksman [68] attributed some microbiological peculiarities of the *Sphagnum* peat to its low pH. Others have postulated the presence of antibacterial substances in such peats so aerobic bacteria can flourish only after antimicrobial compounds of *Sphagnum* degrade sufficiently to lose their bacteriostatic effects.

1.3 Peat as a Source of Flavour

1.3.1 Peat smoking

Peat was originally used widely to fire kilns producing malt for distilleries. With the advent of the railway system, coal and other fuels became available to mainland Scotland and peat use in the Highland/Speyside area reduced [7]. The isolation of the Western Isles, particularly Islay, and Northern Isles continued a lot longer and use of peat remained at a high level. Though the isolation is now not so apparent, the variation in peat use remains. Peat is no longer used as a heat source; it is now used solely as a source of flavour. Burning of peat during kilning of malted barley produces a smoke or "peat reek", constituents of which are adsorbed by the malt and impart distinctive flavour characteristics to the spirit.

1.3.2 The chemical processes of combustion

Emissions from the combustion of any type of fuel depend directly on the chemical composition of that fuel and the combustion conditions. For biomass burning most data are available for wood combustion [69]. The wood combustion process can be summarised as follows:

The first stage of combustion is the heating and evaporating stage. Initially, heat is brought into contact with a piece of wood in the presence of air. Heat causes several reactions. First, it raises the temperature of an area on the wood surface to some depth into the wood. As the wood's surface temperature approaches 100°C, the water in the wood begins to boil, then evaporates. As long as water remains in the wood, its boiling and evaporation rob heat energy from the source, thereby keeping the wood cells from gaining more heat. Moisture must be driven off before combustion can begin, so wood with a high moisture content is hard to ignite. As the water is driven off and the wood temperature rises its constituents start to hydrolyse, oxidise, dehydrate and pyrolyse forming combustible volatiles, tarry substances and highly reactive carbonaceous char

[69]. At the ignition temperature of the volatiles and tarry substances, exothermic reactions known as combustion begin [69]. Gas phase oxidation of the combustible volatiles and tarry products produces flaming combustion. During the flaming combustion, char formation continues until the combustible volatile flux drops below the minimum level required for the propagation of a flame. Solid-phase oxidation of the remaining char produces glowing combustion [70]. When the intensity of the heat flow falls below a minimum level, oxidation of the active char can result in smouldering combustion which is accompanied by the emission of unoxidised volatile products and aerosol particles of the tar in the form of smoke. When peat is burned during malt kilning, flaming combustion is generally avoided and the incomplete smouldering combustion is encouraged in order to produce the compounds responsible for the desired 'peat reek' [12].

1.3.3 Analysis of peat-smoke-derived compounds

Traditionally in the Scotch whisky industry the level of total phenols has been used as a predictor of peated character in malt and spirit. The concentration of phenols in malt is generally measured using steam distillation of whole malt, followed by a colour reaction using one of a variety of chromogens to give a value for total concentration of phenols present. Alternatively, HPLC of the steam distillate from the malt can be carried out in order to quantify the individual phenolic species. HPLC is also used to determine the phenols concentrations in new-make spirit. Unfortunately, there are some shortcomings associated with the present methods of phenol analysis. As a steam distillation step is required for malt analysis, current methods tend to be time consuming. Large volumes of distillate must be collected thus reducing sensitivity. There are specific problems with some of the chromogens used in colorimetric techniques, for example N, N-dimethyl-*p*-phenylendiamine oxalate does not form a colour with para-substituted phenols [71]. An additional problem associated with the HPLC analysis is that *m*- and *p*-cresol co-elute making it impossible to quantify the two compounds individually. Given the shortcomings of current methods, a novel method for the quantification of phenols in malt was proposed.

Solid phase micro extraction (SPME)

SPME, as described in the review by Fritz and Macka [72], initially involves a coated fibre being exposed to a sample or its headspace causing the target analytes to partition between the sample and the coated fibre. In the second step the fibre is transferred to an instrument (normally a GC) for desorption of the extracted analytes. Usually thermal desorption is used, although desorption by means of an appropriate solvent is also feasible. Subsequent chromatographic analysis enables the extracted analytes to be separated from one another and quantified.

In addition to its simplicity, SPME has the advantages that only a very small sample is required and that virtually all of the extracted analytes are introduced into the chromatograph. Sharp peaks are obtained and parts-per-trillion detection limits have been obtained with electron-capture and ion-trap detectors [73]. No liquid solvent is required, so solvent disposal is eliminated. The availability of automated equipment is undoubtedly a key factor in the great popularity of the technique. By using a SPME autosampler, the entire process (sample sorption and subsequent thermal desorption) is performed on-line, operator independent and solvent free.

Quantification in SPME may be based either on an analyte reaching an equilibrium condition between the sample and coated fibre or on non-equilibrium conditions in which a proportional relationship exists between the sorbed analyte and its initial concentration. This is perhaps the only disadvantage of SPME. As it is an equilibrium technique, often only a small fraction of the sample analytes is extracted by the coated fibre. This means that a change in the sample matrix, or any other variable that affects the equilibrium, may affect the quantitative results.

Several experimental parameters can be altered in order to optimise a SPME method: A suitable fibre coating material can enhance selectivity and fibre coating thickness can affect the amount of analyte retained. Agitation of a liquid sample can increase the rate of mass transfer to the fibre. Addition of an inorganic salt to the aqueous sample shifts

the partition equilibrium so that more of the analytes will be extracted. Sample pH can be adjusted to provide better selectivity. Heating liquid samples results in faster diffusion rates of the analytes to the coated capillary surface. Derivatization can be employed to improve extraction and separation of highly polar compounds. Sampling the headspace above a sample (HS-SPME) in an enclosed container allows the analysis of solid samples such as malt and also avoids problems associated with dirty samples where the fibre coating may be plugged by the sample solids when analysed using direct-immersion SPME.

HS-SPME therefore may offer a potentially faster and more convenient alternative to the current methods for measuring phenols in malt. This technique could also be applied to analyse phenols in new-make spirit, where the benefit of its high sensitivity could be utilised.

Solid phase extraction (SPE)

Whilst HS-SPME may offer a useful solution for the analysis of volatile components in the headspace above a complex matrix such as malt, it may not allow the detection of some of the less volatile components detected in peat pyrolysates such as dimethoxyphenols. Therefore, an alternative method considered for analysing compounds adsorbed to malt is SPE. In previous studies, peat-derived compounds have been analysed by steam distillation followed by liquid: liquid extraction [9,74]. However, compared to liquid: liquid extraction, SPE has several advantages: SPE is faster and requires less manipulation. It also requires much less organic solvent and provides higher concentration factors.

As described by Fritz and Macka [72], typically in SPE, an aqueous sample is passed through a small tube filled with porous solid particles. The extracted analytes bind to the solid particles and can subsequently be eluted using a small volume of an organic solvent. A portion of the eluate can then be analysed by gas or liquid chromatography. The most common type of solid-phase extraction is reversed-phase SPE. Here the goal is to isolate relatively non-polar organic analytes from a predominantly aqueous sample. This

requires the use of relatively hydrophobic adsorbent particles such as silica with bonded octadecylsilane groups, porous organic particles such as highly cross linked polystyrene, or various types of activated carbon or graphitized carbon materials. One such sorbent is Strata-X (Phenomenex), which has a styrenic skeleton and is modified with a pyrrolidone group. The retention mechanisms of this sorbent are hydrophobic, hydrogen-bonding and aromatic [75]. Therefore Strata-X is useful for the extraction of polar to non-polar analytes. This would appear to make this sorbent appropriate for the analysis of the range of compounds produced during peat pyrolysis.

1.3.4 Smoke composition

Many peat-smoke-derived aroma compounds have previously been reported in peated malt ranging from simple hydrocarbon tars to complex heterocyclic compounds [74,76,77]. These included a range of phenolic compounds and carbohydrate-derived compounds as well as several nitrogen-containing compounds.

Phenols

Investigations carried out on wood smoke condensate, where measures were taken to remove non-phenolic compounds, showed that the essential smoke odour was retained [78]. It must also be noted that during the investigation of wood smoke constituents it was found that the participation of carbonyl, acidic and pyrazine compounds was necessary to obtain agreeable flavour [78-80]. Nevertheless, as mentioned previously, traditionally in the Scotch whisky industry the level of total phenols is used as a predictor of peated character. Aroma descriptions of some phenols found in peated malt are listed in Table 1.1.

Table 1.1. Aroma descriptions of selected phenols.

Compound	**Aroma**[a]
Phenol	strongly phenolic, medicinal, antiseptic
m-Cresol	phenolic
o-Cresol	phenolic
p-Cresol	powerful cresylic
4-Ethylphenol	phenolic, aromatic, slightly spicy
Guaiacol	aromatic, phenolic, burnt; woody, bacon, savoury, smoky and medicinal
Methylguaiacol	spicy, medicinal, vanilla, clove-like with phenolic nuances
Ethylguaiacol	spicy, smoke-like; on dilution bacon-like, medicinal, woody and sweet vanilla nuances
4-Vinyl guaiacol	aromatic, spicy, somewhat phenolic
Syringol	aromatic, phenolic, somewhat spicy, smoky and bacon-like sweet, medicinal, creamy, meaty, vanilla, spice

[a] Aroma descriptions taken from the Bacis 98 database of flavour (raw) materials [81].

Given the different aromas described in Table 1.1, different flavour notes found in peated spirits will depend not only on the type of compound present but also on their relative proportions. It is also important to note that not all of the phenolic compounds occur at high enough levels in new-make spirit to impart a distinctive aroma. For example,

phenol is normally present in the highest concentration [9] but it is present only at sub-threshold levels (threshold approximately 19 ppm) and is not itself of sensory significance whereas the aroma potential of some other phenols, particularly *p*-cresol and guaiacol, may be greater [82]. It is possible therefore, that two peats of different chemical composition could produce peated malts which by classical analyses, determining only total phenols, would give the same rating for phenolic content. However, the spectrum of phenols, and indeed other flavour compounds, in the two malts could be very different, resulting in an entirely different spirit flavour.

It has also been noted that in whiskies made using relatively low amounts of peated malts, there is not always a correlation between the amount of peated malt used and resultant peat associated flavour [82]. This may be due to there being other sources of phenolic compounds. For example, during mashing, Steinke and Paulson [83] demonstrated that compounds such as 4-vinylphenol and 4-vinylguaiacol are formed by thermal decarboxylation of *p*-coumaric acid and ferulic acid. Subsequent yeast and particularly bacterial action converts these intermediates into 4-ethylphenol and 4-ethylguaiacol [84].

Carbohydrate derivatives

Compounds such as furfural and 5-hydroxymethylfuran have previously been identified in peated malts though the origin of these compounds was not clear in this study [74]. Nevertheless, compounds in this class are known to possess caramel and burnt flavour notes so could contribute to the aroma of peated malts [85]. In wood smoke preparations it has been reported that furan and pyran derivatives are responsible for the softening of the heavy aromas associated with phenolic compounds [86].

Nitrogen-containing compounds

Five classes of nitrogen compound have also been detected in whisky at low levels: aliphatic amines, thiazoles, pyrazines, pyridines and quinolines [87]. The origin of these compounds in whisky is not certain, though pyridine itself has been detected in the pyrolysates of peat [88]. Also, pyrazine compounds have previously been found in

peated malt though similar compounds were also found in unpeated malt [76]. This implies that pyrazine compounds in peated malt result, at least in part, from raw malt.

Pyridines, pyrazines and thiazoles are known to have low odour thresholds and so could theoretically contribute to spirit aroma [89]. For example, the pyrazine compounds seem to participate in the aroma of alcoholic beverages with flavours such as "burnt", "roasted" and "nutty" which could contribute to the peaty aroma [90]. The impact of the pyridines on flavour may be inhibited in mature spirit as pyridines have relatively high pK_a values and the pH of the spirit is relatively low therefore the concentration of pyridines released into the headspace is low [91].

1.3.5 Factors affecting smoke composition

There are four main factors that influence smoke composition: composition of combustion materials, pyrolysis temperature, moisture content and air supply.

Composition of combustion material

An important point raised by the study of wood smoke derived from different sources is the difference between hard wood (angiosperm) and soft wood (gymnosperm) derived smoke. In this regard, particular attention has been paid to the different methoxyphenols produced. Methoxyphenols are derived from lignin and, as mentioned previously, the lignins found in hardwood and softwood are different. Softwood gives rise to guaiacyl compounds, whereas mainly syringyl compounds are formed from hardwood [92-94].

Compounds released from the burning of various Scandinavian forest plant materials have been analysed for the types of methoxyphenols released using GC-MS [95]. Wood, twigs, bark and needles from conifers emitted guaiacyl compounds in similar proportions. Grass, heather and birchwood additionally released the corresponding syringyl compounds. Furthermore, the proportion of syringyl compounds was very different in different angiosperms, ranging from 80% for birchwood down to 30% for grass. As would be expected from their lack of lignin, when moss and lichens were burnt there was

found to be no methoxyphenols released. These observations suggest that variations in peat constituents are likely to result in differences in smoke composition.

Pyrolysis temperature

In a previous study, the main component parts of wood (lignin, hemicellulose and cellulose) were pyrolysed at various temperatures. Temperature of pyrolysis was found to have an important effect on the proportions of breakdown products [96]. In the case of lignin, at 400 °C the pyrogram revealed mainly the presence of different primary pyrolysis products such as vanillins and guaiacols. At 600 °C, particularly vanillin derivatives were converted into various catechols and phenols. At higher temperatures (800 °C and 1000 °C) the relative mass portions of aromatic hydrocarbons, such as benzene and naphthalene and other phenols such as phenol and various methylphenols, formed as a result of further reaction of primary pyrolysis products, were significant. The degradation compounds from the carbohydrate components of wood, cellulose and hemicellulose, formed during pyrolysis over the same temperature range were also assessed. Here, whilst anhydrosugars were the main products at low temperatures, as the temperature approaches 1000 °C, more volatile compounds, such as acetic acid and 1-hydroxy-2-propanone, were increasingly evident. Additionally, aromatic hydrocarbons were also evident at temperatures above 600 °C.

Moisture content of combustion material

During wood combustion, if the moisture content is high, an appreciable amount of energy is necessary to vaporise the water. This reduces the heating value of the wood as well as decreasing combustion efficiency, which in turn increases smoke formation [97]. On the other hand, wood with a low moisture content burns faster, eventually causing oxygen-limited conditions that lead to incomplete combustion with increased wood smoke particle formation.

Liquid smoke produced from the pyrolysis of wood showed that the highest yield in total compounds was obtained from samples with low moisture content (which caused the highest temperatures and shortest pyrolysis times) [98]. Some groups of compounds,

such as pyran and furan derivatives, were not affected by moisture content as much as others, such as aldehydes and diketones. Some compounds, including phenols such as vinylguaiacol and vinylsyringol, were found to be prominent at medium moisture levels.

Air supply

Combustion can occur if an oxidising agent (air) and an ignition source are present and provided that they are heated to their piloted ignition temperatures [99]. Therefore the amount of air present during the smoke generation is considered to be influential on the characteristics of the smoke produced [100,101]. Complete combustion requires an adequate air supply while limiting the air supply inhibits complete combustion. Incomplete combustion yields the desired flavour compounds in the form of smoke, though too little air will not support combustion and result in the fire dying out.

1.5 Aims and Objectives

Peat used in the production of peated malt for the Scotch whisky industry is sourced from various locations around Scotland. Past research, summarised in the introduction, suggests that peat composition is likely to vary from location to location. Such variation could ultimately impact on spirit flavour. However, no research has been carried out to date in this area. Improved understanding of compositional variation and potential flavour impact would be useful to the industry should malt producers change their peat source, through choice or necessity, in the future.

The aims of this research are outlined below:

- To determine the compositional differences between peats from different geographical locations in Scotland.
- To examine whether or not differences in peat composition are transferred to malt and subsequently to new-make spirit.
- To determine whether or not peat related differences in composition have an impact on spirit flavour.
- To study the relevance of any observed flavour differences in actual distillery situations.

This will be achieved by the collection of representative peat samples from a number of locations currently used by the Scotch whisky industry. Methods will be developed for the compositional analysis of these peats. The peat samples will then be used to produce peated malt in the laboratory. This will require the development of novel methods both for the production and analysis of this malt. The malt will then be used to produce spirit, again under carefully controlled laboratory conditions. Sensory and analytical evaluation of the resulting spirits will allow the impact of peat source on both spirit composition and flavour to be determined. Finally the relevance of any observed flavour differences will be examined through comparison with industrial peated spirits from various distilleries.

Chapter 2: Materials, Methods and Method Development

2.1 Samples

2.1.1 Peat sampling from different geographical regions

Peat sampling sites

Peat was sampled from six locations representing the sites used by the peat suppliers to the malting industry in Scotland (Fig. 2.1 and Table 2.1).

Figure 2.1. Peat sampling sites. **1.** Hobbister hill, by Kirkwall, Orkney, **2.** St Fergus, **3.** Tomintoul, **4.** Castlehill, Islay, **5.** Glenmachrie moss, Islay, **6.** Gartbreck moss, Islay.

Table 2.1. Descriptions of peat sampling sites.

Site name	Abbreviation	Location	OS National Grid reference	Deposit type
Castlehill	P	Islay	NR3650	Blanket bog
Gartbreck moss	B	Islay	NR2858	Basin/ valley bog
Glenmachrie moss	L	Islay	NR3350	Basin/ valley bog
Hobbister Hill	H	Orkney	HY3806	Blanket bog
St Fergus	N	Aberdeenshire	NK0553	Basin/ valley bog
Tomintoul	T	Speyside	NJ2020	Basin/ valley bog

Site name	Approximate extraction site area (hectares)[a]	Extraction site depth (metres)[a]	Peat cutting method[b]
Castlehill	85	0.4–7.4	Extruded
Gartbreck moss	12	1.8-9.1	Extruded
Glenmachrie moss	12	1	Banks
Hobbister Hill	(less than) 12	0.6–2.4	Banks
St Fergus	120	1–3	Extruded
Tomintoul	20	1.2–2.3	Extruded

[a] Approximate extraction site area and depth data was supplied by site operators.

[b] Extruded peat is forced out by machine from a particular depth below the bog surface to produce long sausage shaped lines of peat on the bog surface. Bank-cut peat is cut along banks in blocks either by hand or machine from the surface down to a predetermined depth.

Transect sampling

The aim of this sampling programme was to collect sets of samples which could be used to compare intra-site variation of peat chemical composition with inter-site variation. Therefore, it was necessary to collect a representative set of randomly collected samples from each site. Given the limited resources available, randomly placed transect lines were used to ensure that a representative area of each site was sampled. From each

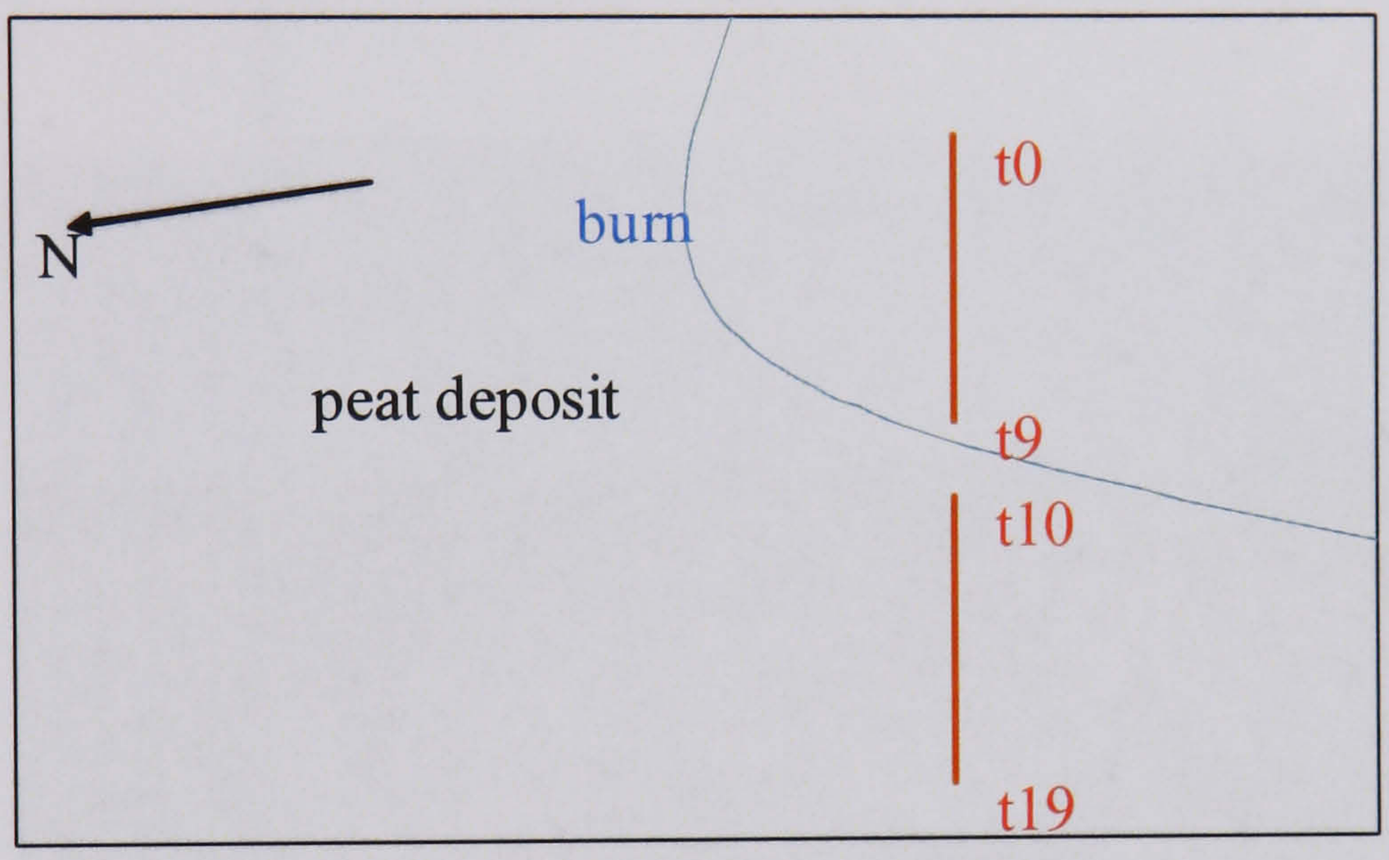
sampling site, 20–24 samples of approximately 1–3 kg were manually extracted from approximately the same depth (30–50 cm) at equal intervals along 1 to 3 transect lines. Considering each site as a square, the aim was for the total transects length to cover approximately 50% of the width of the site. The appropriate lengths of the transect lines were generally estimated in relation to the boundaries of the extraction site by eye. In cases where the boundaries were poorly defined (Gartbreck, Glenmachrie and Hobbister Hill), the appropriate length of transect line was estimated in relation to the approximate current extraction site area data supplied by the site operator (Hobbister Hill was assumed to be 12 hectares). The length of transect line was approximated by the number of paces and this value was converted to metres. Details of the sampling protocol for each site are provided in Table 2.2.

The sampling protocols were adapted according to site management methods. For those sites where peat is extruded by machine, samples were taken along one or, where necessary, two randomly placed line transects (Figs. 2.2–2.5). A total of 20 samples were taken from each of these sites. For those sites where peat is cut along banks, samples were taken from line transects divided among randomly selected banks in order to cover an appropriate distance (Figs. 2.6–2.7). 24 samples were taken from these sites to get a representative number from each peat bank sampled.

For sample labels, the uppercase letter indicates the site as per the abbreviations in Table 2.1. A lower case t indicates a transect sample (t) and the letters a, b or c the specific transect where more than one was taken. The number indicates the sample number; thus, Tt16 indicates sample 16 of a transect taken at Tomintoul.

Table 2.2. Protocols for transect line sampling of peat sites.

Site	No. of transects	Length of transect (m)	Total length of transect (m)
Castlehill	2	225	450
Glenmachrie	3 (ta, tb, tc)	ta:56, tb:56, tc:56	170
Gartbreck	1	170	170
Hobbister Hill	3(ta, tb, tc)	ta: 60, tb: 45, tc: 53	160
St. Fergus	1	380	380
Tomintoul	1	190	190

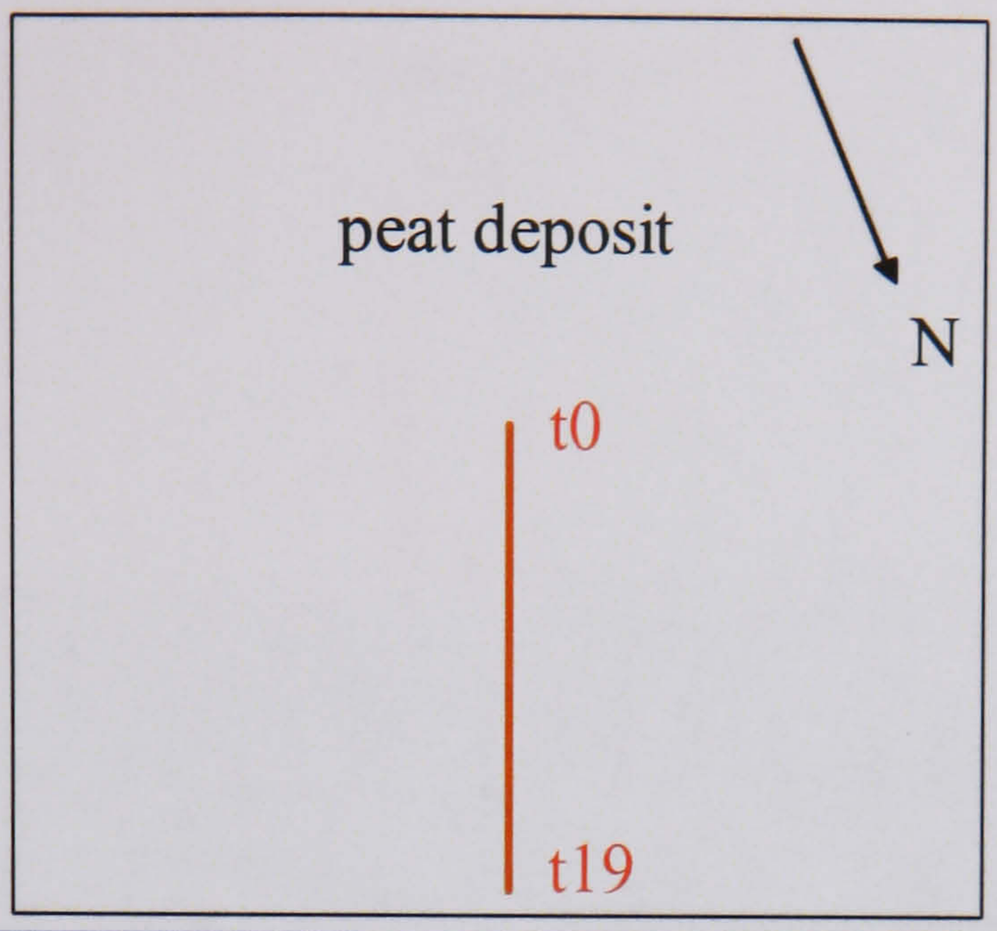


a

b

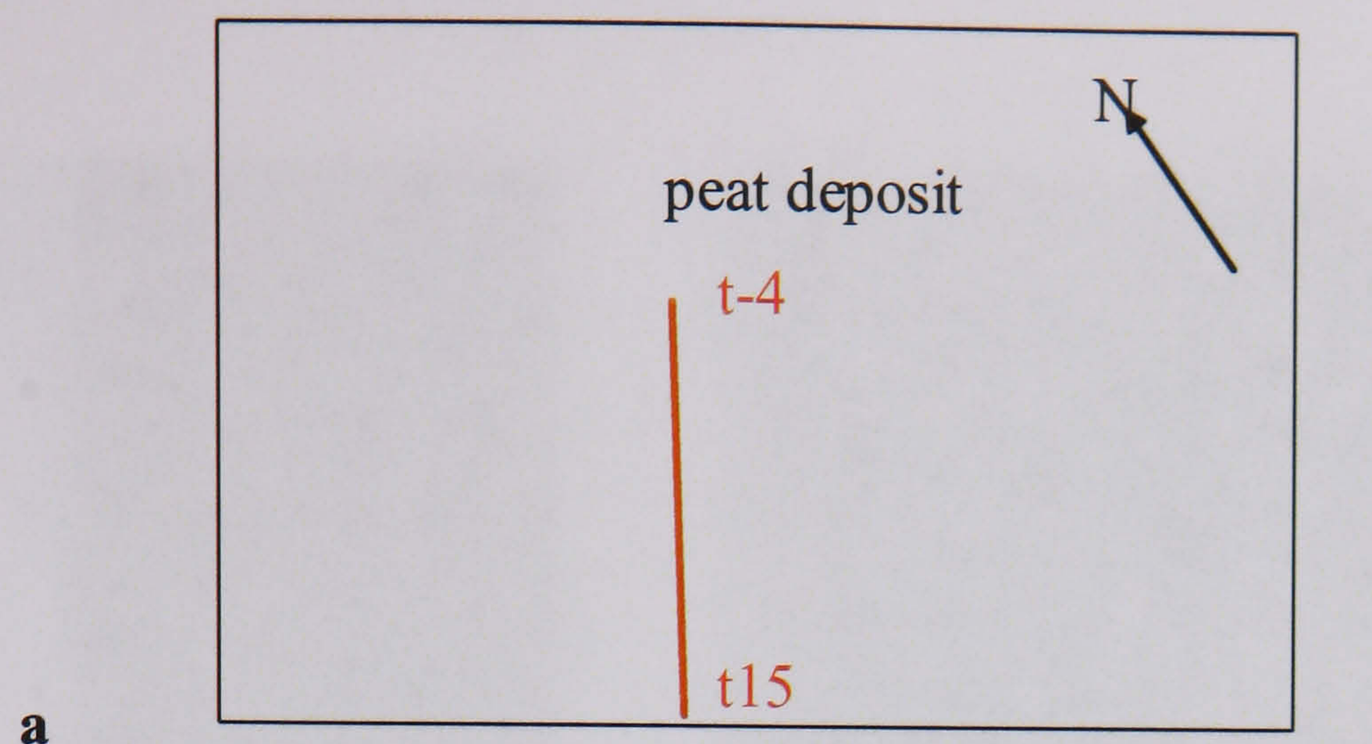
Figure 2.2. a: Schematic of Castlehill sampling site (red line indicates site of transect line), b: view from sample site t0 towards t19.

a



b

Figure 2.3. a: Schematic of Gartbreck sampling site (red line indicates site of transect line), b: view from sample site t19 towards t0.



a

b

Figure 2.4. a: Schematic of St Fergus sampling site (red line indicates site of transect line), b: view from sample site t0 towards t15.

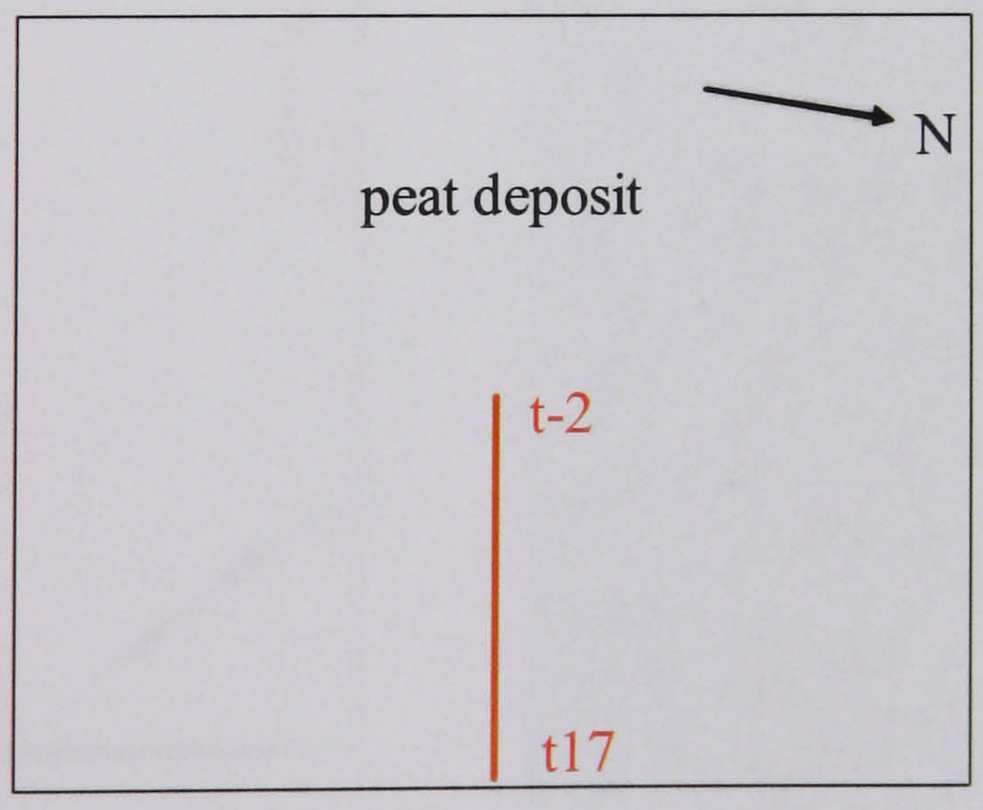
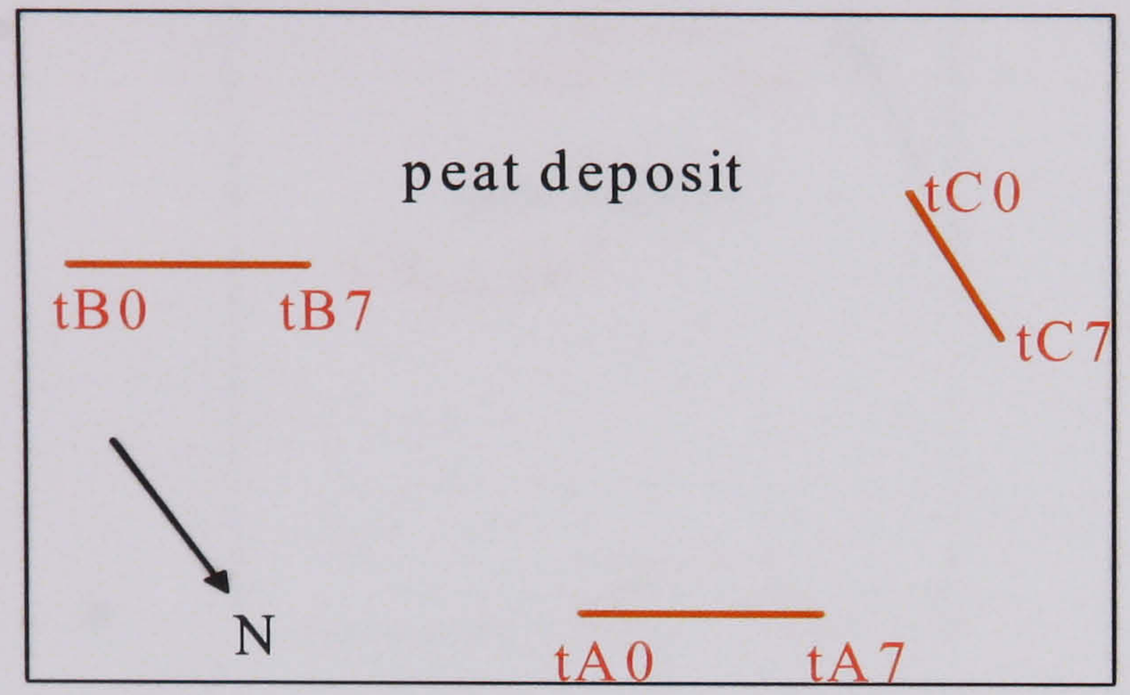


Figure 2.5. Schematic of Tomintoul sampling site (red line indicates site of transect line).

a

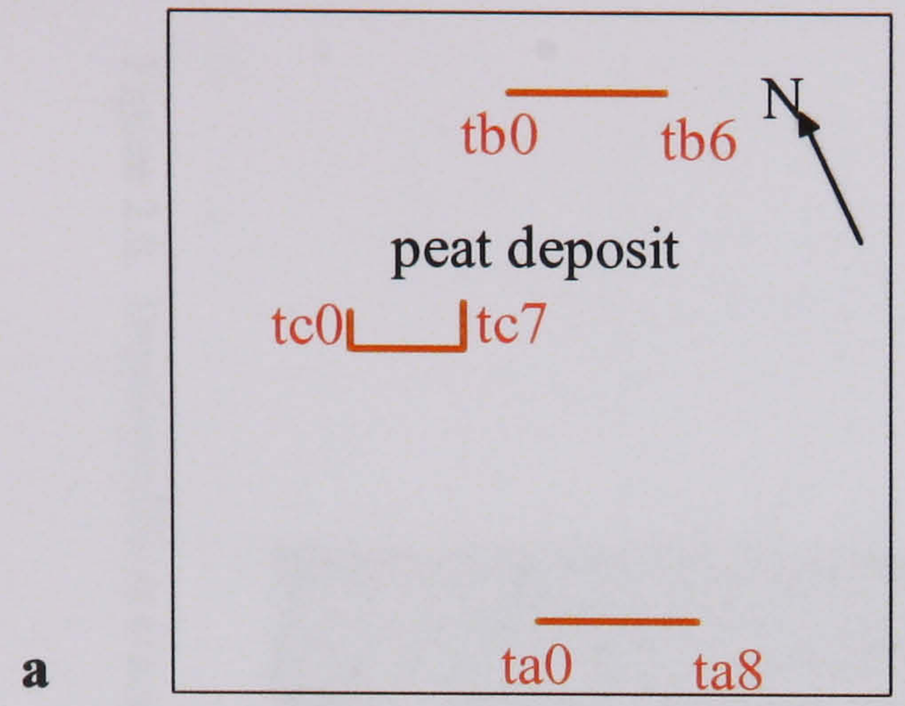


b

c

d

Figure 2.6. a: Schematic of Glenmachrie sampling site (red line indicates site of transect line), b: view from sample site ta0 towards ta7, c: view from tb0 on left to tb7 on right of peat face, d: view from tc0 towards tc7.



a

b

c

d

Figure 2.7. a: Schematic of Hobbister hill sampling site (red line indicates site of transect line), b: view from sample site ta0 towards ta8, c: view from tb0 on left to tb6 on right of peat face, d: tc.

Depth profiles

At sites where peat was cut along banks (Hobbister Hill and Glenmachrie) it was also possible to take samples from different depths to assess the effect of depth on peat composition (Figs. 2.8 and 2.9). The positions of these depth profiles were chosen points where the peat banks were at their deepest: Hobbister Hill- approximately 20 m from start of tb, Glenmachrie- approximately 18 m from start of ta. At Hobbister Hill, five samples were taken, from just below the surface to the bottom of the bank at 30 cm intervals (p0–p4). At Glenmachrie four samples were taken, from about 10 cm below the surface to about 30 cm from the bottom of the bank at 25 cm intervals (p0–p3).

Figure 2.8. Depth profile at Glenmachrie moss.

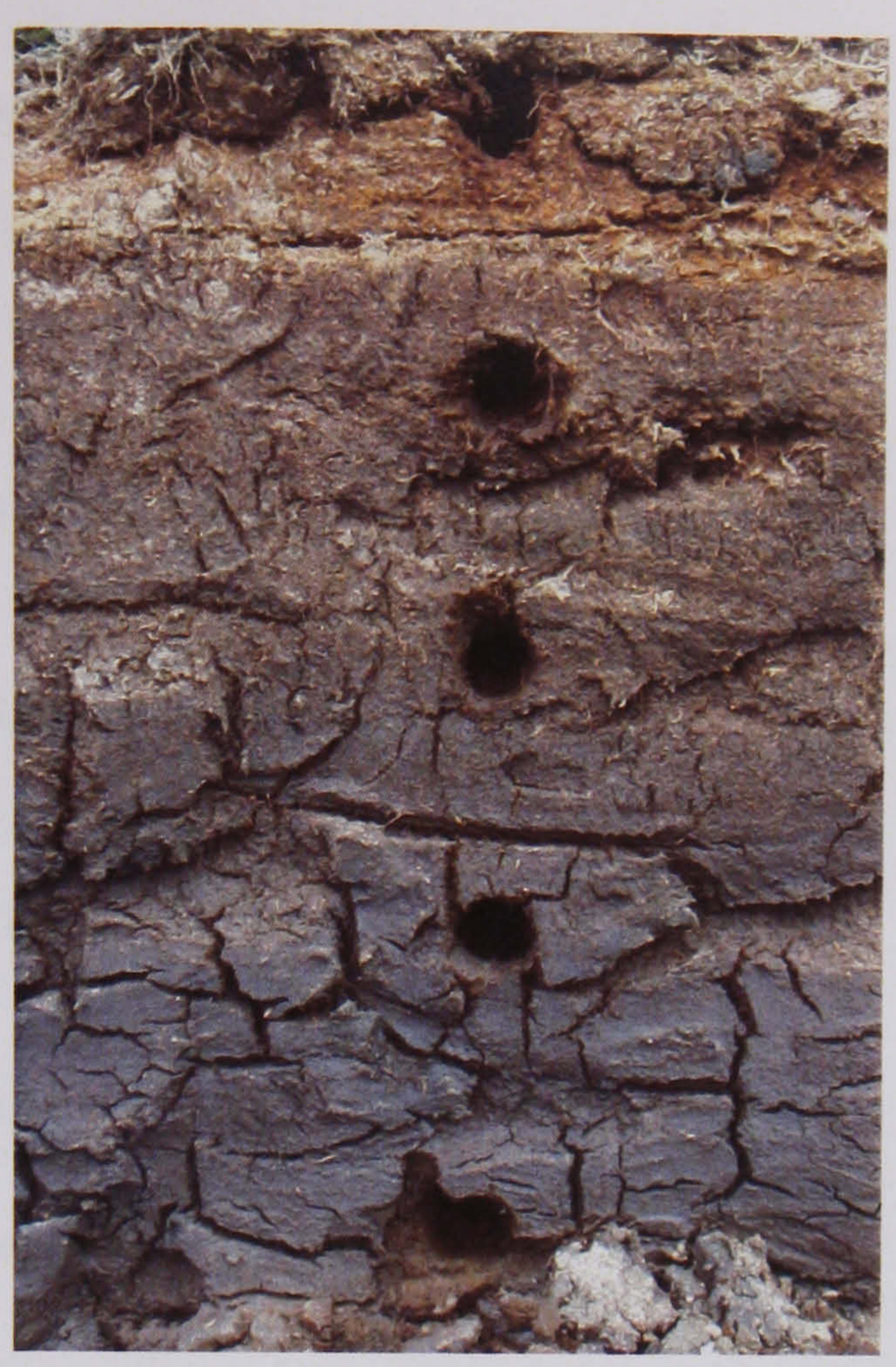


Figure 2.9. Depth profile at Hobbister Hill.

Industrial samples

To assess how well transect samples represented commercially cut peat, a sample of commercially cut peat was taken either from beside the peat deposit or from the storage shed at each peat sampling site. Industrial samples are subsequently referred to using the appropriate abbreviation from Table 2.1 and the letters "ind", e.g. Nind is an industrial sample from St Fergus.

Sample storage

After collection, all peat samples were stored, sealed in polyethylene bags, in a cold store at approximately 5 °C until all sites had been sampled.

Peat moisture content

Four samples from each set of transect samples were subjected to moisture content analysis using an oven drying method. Approximately 5 g of sample was weighed out into a previously weighed lidded vessel. The samples were then oven dried in these vessels (with the lids off) at 105 °C for 4 h. The lids were put on the vessels and the samples were then transferred to a dessicator. The samples were left in the dessicator for 25 min to allow them to reach room temperature and were then reweighed. The difference between the wet and dry weights gave the peat moisture content. This value was reported as a percentage of the original wet weight. Table 2.3 shows the mean moisture content of each peat.

Table 2.3. Mean moisture content of peat from six sources.

Peat source	Moisture content (%)	Standard deviation
Gartbreck	86.1	1.6
Hobbister Hill	85.7	3.2
Glenmachrie	88.6	1.4
St Fergus	77.1	22.8
Castlehill	88.3	2.6
Tomintoul	82.7	9.1

Generally, the samples were all found to contain similarly high moisture contents. The low mean value for St Fergus was due to one measured sample (Nt15) having an unusually low moisture content (43.0%). Removal of this sample increased the St Fergus mean to 88.5%.

Peat sample drying

The collected peat samples contained a high moisture content. Therefore, as occurs in industry, to allow the samples to be stored and to make subsequent sample handling

easier, they were dried. Once sampling was completed, all samples were transferred to greenhouses for drying. Sample moisture content was periodically measured using a Protimeter moisture meter (Protimeter plc., Marlow, Bucks, UK, SL7 1LW). Once samples reached a moisture content of below 20%, they were sealed in polypropylene buckets and stored outside at ambient temperature.

Peat grinding

Initially, dried samples were ground roughly using a Bosch AXT RAPID 180 garden shredder (Robert Bosch Limited, Uxbridge, Middlesex, UK, UB9 5HJ). Representative sub-samples were then obtained from each sample using a sample divider (Endecotts Limited, London, UK, SW19 3TZ), frozen in liquid nitrogen and ground finely using a SPEX 6700 freezer mill (Glen Creston Limited, Stanmore, Middlesex, UK, HA7 1BU).

2.1.2 Malt sampling

Samples of commercially produced peated malt were provided by various peated malt producers who use peat from the sources that were sampled (Fig. 2.10 and Table 2.4).

Figure 2.10. Map of peated malt producers. **1.** Highland Park Distillery, **2.** Glen Ord Maltings, **3.** Bairds Maltings, **4.** Muntons Maltings, **5.** Port Ellen Maltings, **6.** Bowmore Distillery, **7.** Laphroaig Distillery.

Table 2.4. Peat source of peated malt producers.

Peated malt producer	**Peat source**
Highland Park Distillery	Hobbister Hill
Glen Ord Maltings	St Fergus
Bairds Maltings	St Fergus
Muntons Maltings	Tomintoul
Port Ellen Maltings	Castlehill
Bowmore Distillery	Gartbreck moss
Laphroaig Distillery	Glenmachrie moss

All peated malt samples comprised Optic barley except the Glen Ord sample which was Decanter.

2.1.3 New-make spirit sampling

Samples of peated new-make spirit were provided by various distillers who use peat from the sources that have been sampled as well as some distillers who used other sources of peat (Table 2.5). One unpeated sample was also provided.

Table 2.5. New-make spirit samples supplied by industry.

Sample ID	Peat source	Distillery[a]
caith a	Caithness	Highland Park
caith b	Caithness	Highland Park
invernesshire	Invernesshire	Jura
islay a	Islay (Glenmachrie)	Laphroaig
islay b	Islay (Castlehill)	Jura
islay c	Islay (Castlehill)	Ardbeg
islay d	Islay (Castlehill)	Diageo a
islay e	Islay (Castlehill)	Diageo b
islay f	Islay (Gartbreck)	Bowmore
orkney a	Orkney	Highland Park
orkney b	Orkney	Highland Park
st f a	St Fergus	Knockdhu
st f b	St Fergus	Diageo c
st f c	St Fergus	Diageo d
st f d	St Fergus	Diageo e
st f f	St Fergus	Jura
st f g	St Fergus	Dalmore
sweden a	Sweden	Mackmyra
sweden b	Sweden	Mackmyra
up	unpeated	Diageo f

[a] Letters indicate samples from different distilleries owned by Diageo.

2.2 Chemicals and solvents

Authentic samples of the following phenols were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, UK, SP8 4XT): phenol, *o*-cresol, *p*-cresol, guaiacol, 4-ethylphenol, *m*-cresol, 4-methylguaiacol, 4-ethylguaiacol and 2, 3, 5 trimethylphenol.

Authentic samples of the following esters were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, UK, SP8 4XT): ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexanoate, ethyl tetradecanoate ethyl hexadecanoate and methyl octadecanoate.

Authentic samples of naphthalene-d8 and pyridine-d5 were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, UK, SP8 4XT).

Acetic acid, acetonitrile and dichloromethane (DCM) were purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland, UK, EH43 6AU). Ethanol (EtOH) was purchased from McQuilkin & Co. (College Milton North, East Kilbride, UK, G74 5HD). Ultra High Quality (UHQ) water produced using an ELGA LabWater Purelab UHQ 11 purification system (ELGA LabWater Global Operations, UK., HP14 3BY).

2.3 Analysis of Peat Samples using Fourier Transform Infrared Spectroscopy (FT-IR)

2.3.1 Sample preparation

Zero point five gram samples (± 0.0005 g) of each finely ground peat were suspended in 4 mL UHQ, mixed, and 3 aliquots (10 µL) were placed on a 96-well aluminium plate and dried at 50 °C for 30 min.

2.3.2 Analytical instrumentation

The plate was loaded onto a motorised micro plate module HTS-XT™ attached to an Equinox 55 module (Bruker Optics Ltd., Coventry, UK, CV4 9GH). The motorised module of this instrument introduces the plate into the airtight optics of the instrument, in which tubes of desiccant are contained to remove moisture [102]. An MCT (mercury-cadmium-telluride) detector cooled with liquid N_2 was employed for collection of IR spectra in reflectance mode. Spectra were collected in triplicate (giving a total of nine spectra for each sample) over the wavelength range of 4000 to 800 cm^{-1} under the control of a computer programmed with Opus 4, operated under MS Windows 2000. Spectra were acquired at a resolution of 4 cm^{-1}, and 64 spectra were co-added and averaged to improve the signal to noise ratio. The collection time for each spectrum was approximately 60 s and the spectra were displayed in terms of absorbance.

In order to account for unavoidable baseline shifts when collecting spectra in reflectance mode, the baseline was corrected using rubber banding (provided by the instrument manufacturers) and each spectrum was then scaled linearly so that the minimum absorbance was set to 0 and the maximum to 1.

2.4 Analysis of Peat Samples using Curie Point Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

2.4.1 Sample preparation

Three hundred milligram samples of finely ground peat were suspended in 5 mL UHQ water and homogenised. One or two drops of peat suspension were applied onto a ferromagnetic wire using a micro capillary tube. The Curie point temperature of the wire was 610 °C. Wires were dried at room temperature whilst being rotated constantly to ensure an even coating of the sample around the wire. All samples were analysed in duplicate.

2.4.2 Analytical instrumentation

Pyrolysis was performed using a Horizons instruments Curie point pyrolyser (Horizon Instruments Ltd., Heathfield, East Sussex, UK, TN21 8AW). Each prepared sample was connected to the probe of the pyrolyser and manually inserted into the radio frequency (RF) coil. The pyrolyser was then allowed to equilibrate for a few minutes. Pyrolysis heating was initiated and held for 3 s during which time the wire reached its Curie point temperature (switching from ferromagnetic to paramagnetic). The pyrolysate then passed onto the column within the gas chromatograph.

GC-MS was performed on a Hewlett-Packard 5890 series II gas chromatograph coupled to a 5971 mass spectrometer (Agilent Technologies UK Limited, Stockport, Cheshire, UK, SK8 3GR). The column used was a 30 m x 0.25 mm ZB5ms capillary column with a film thickness of 0.5 μm (Phenomenex, Macclesfield, Cheshire, UK, SK10 2BN). Analysis was carried out in split mode. The carrier gas was He, the head pressure was 11 psi (giving a flow of 1 mL min^{-1} at 100 °C) and the split flow was 5 mL min^{-1}. The inlet temperature was maintained at 250 °C throughout the analysis. The initial oven temperature was 40 °C, held for 1 min, increasing to 280 °C at 6 °C min^{-1} with a final hold time of 9 min. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans per second. The GC line temperature was maintained at 310 °C throughout the analysis.

All peak quantifications were made on integrated single ion peaks to diminish co-elution problems.

Compound identities were obtained initially by comparison of their mass spectra with those found in the National Institute of Standards and Technology (NIST) library (version 2.0) (Gaithersburg, MD 20899-1070, USA). To support the identification of compounds by MS, estimated retention indices were calculated for all compounds using the assumption that when carrying out temperature-programmed GC-MS analysis, as was the

case here, retention indices can be related to retention times on an approximately linear scale [103]. Therefore, literature retention index data (which had been obtained using *n*-alkanes as reference substances) for compounds which were identified here by MS with a high degree of certainty were plotted against their experimental retention times recorded in this work. A high R^2 value for the resultant linear equation supported the identification of these compounds. Subsequently, this linear equation was was used to calculate estimated retention indices for all analysed compounds and these values were compared with literature retention index data. A good match with literature retention index data was used to support the identification of compounds by MS.

2.4.3 Method development

Pyrolysis temperature

To determine the effect of temperature on the products of peat pyrolysis, wires with various Curie point temperatures (358 °C, 510 °C, 610 °C and 770 °C) were used to analyse a single peat sample. The compounds analysed were the 122 compounds listed in Table 2.6 (Appendix A). The estimated retention indices listed in Table 2.6 were calculated as described in Chapter 2.4.2 and Fig. 2.11 shows the plot of literature retention index data for compounds identified here with a high degree of certainty by MS versus experimental retention time data.

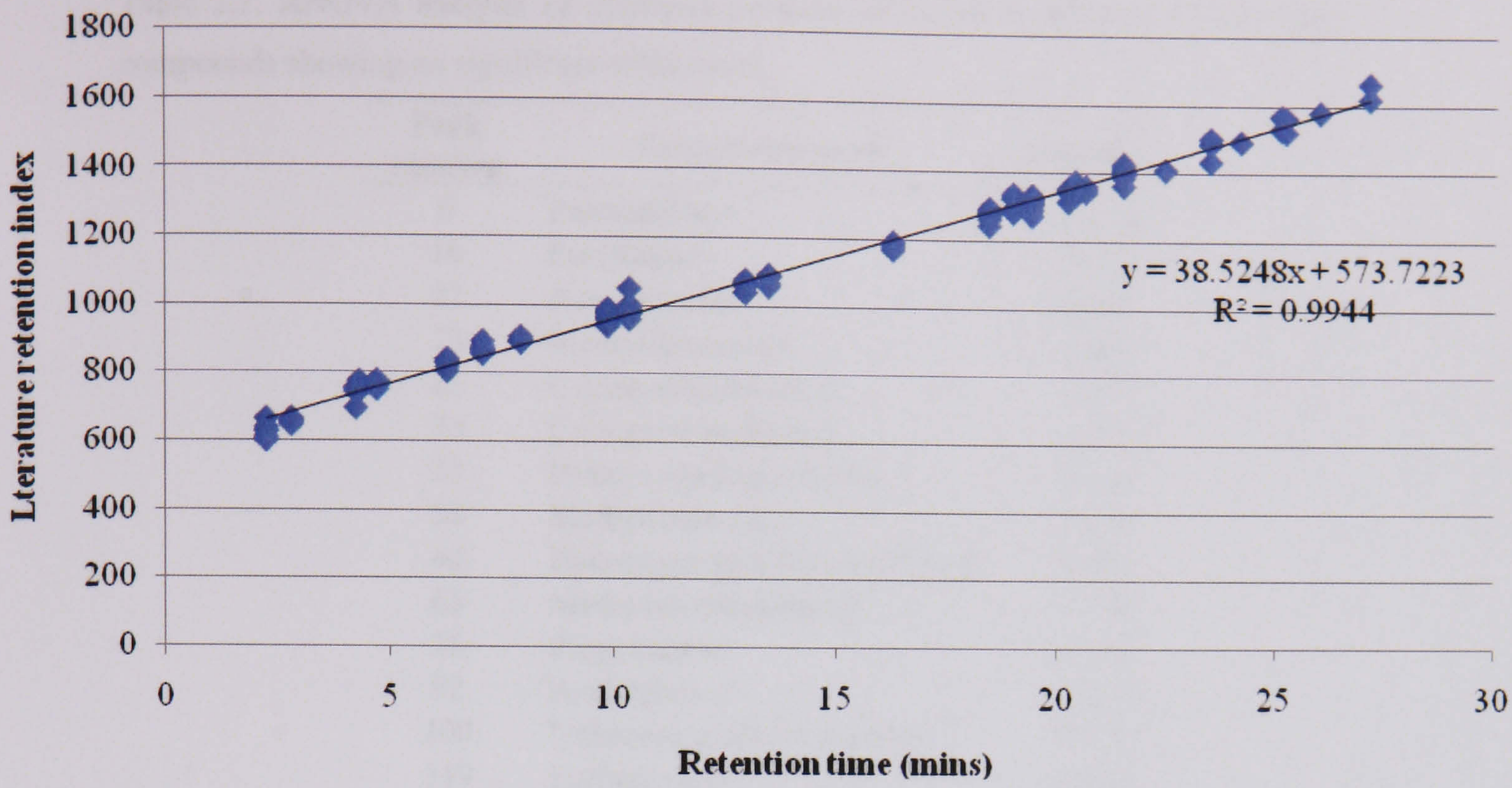


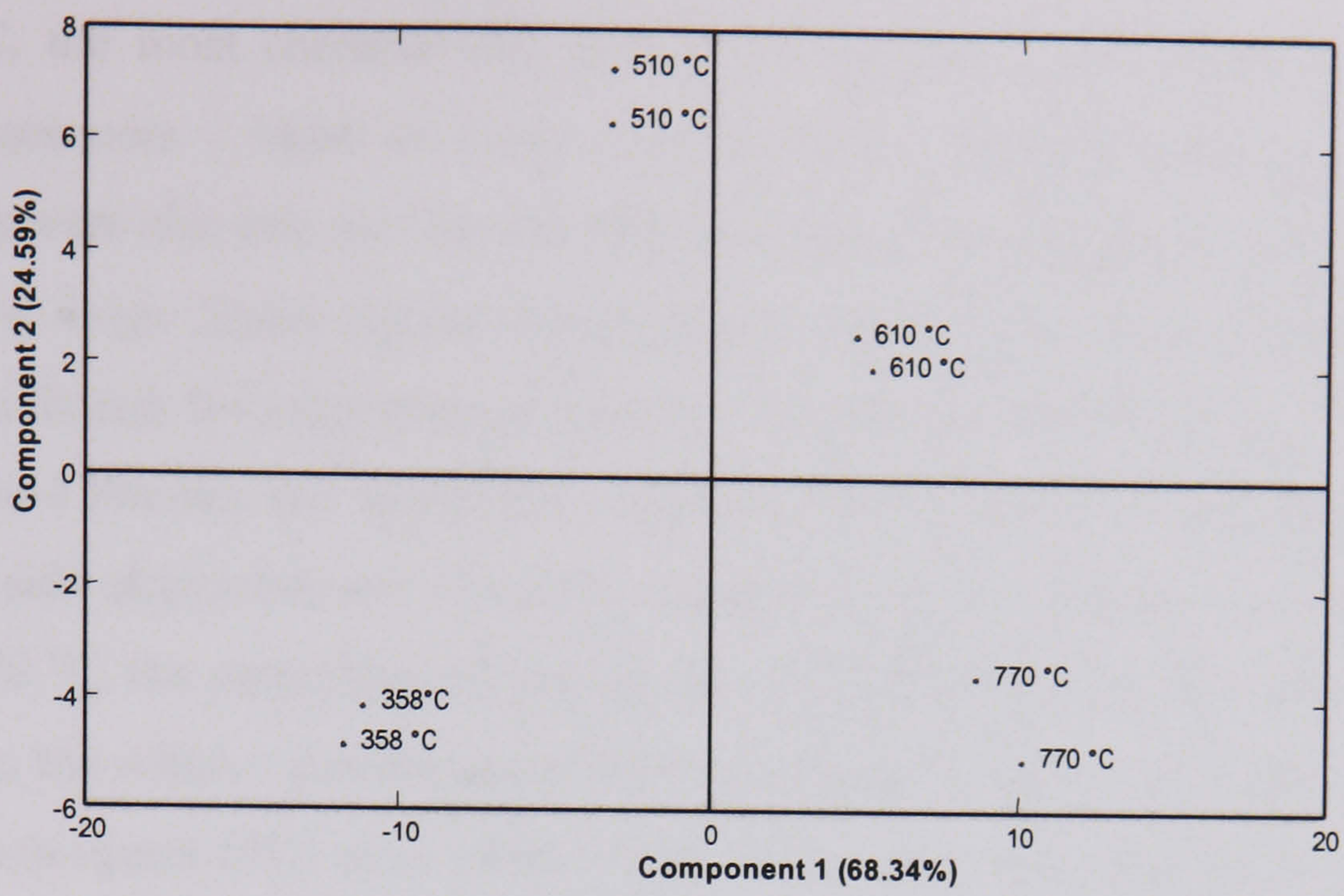
Figure 2.11. Plot of literature retention index data for compounds identified in peat pyrolysates with a high degree of certainty by MS versus experimental retention time data.

The peak area data for these compounds were normalised as a percentage of total peak area and then analysed by analysis of variance (ANOVA) (as described in Chapter 2.15.1) using temperature as a factor. In this way, 16 compounds not showing significant differences between temperatures were removed (Table 2.7).

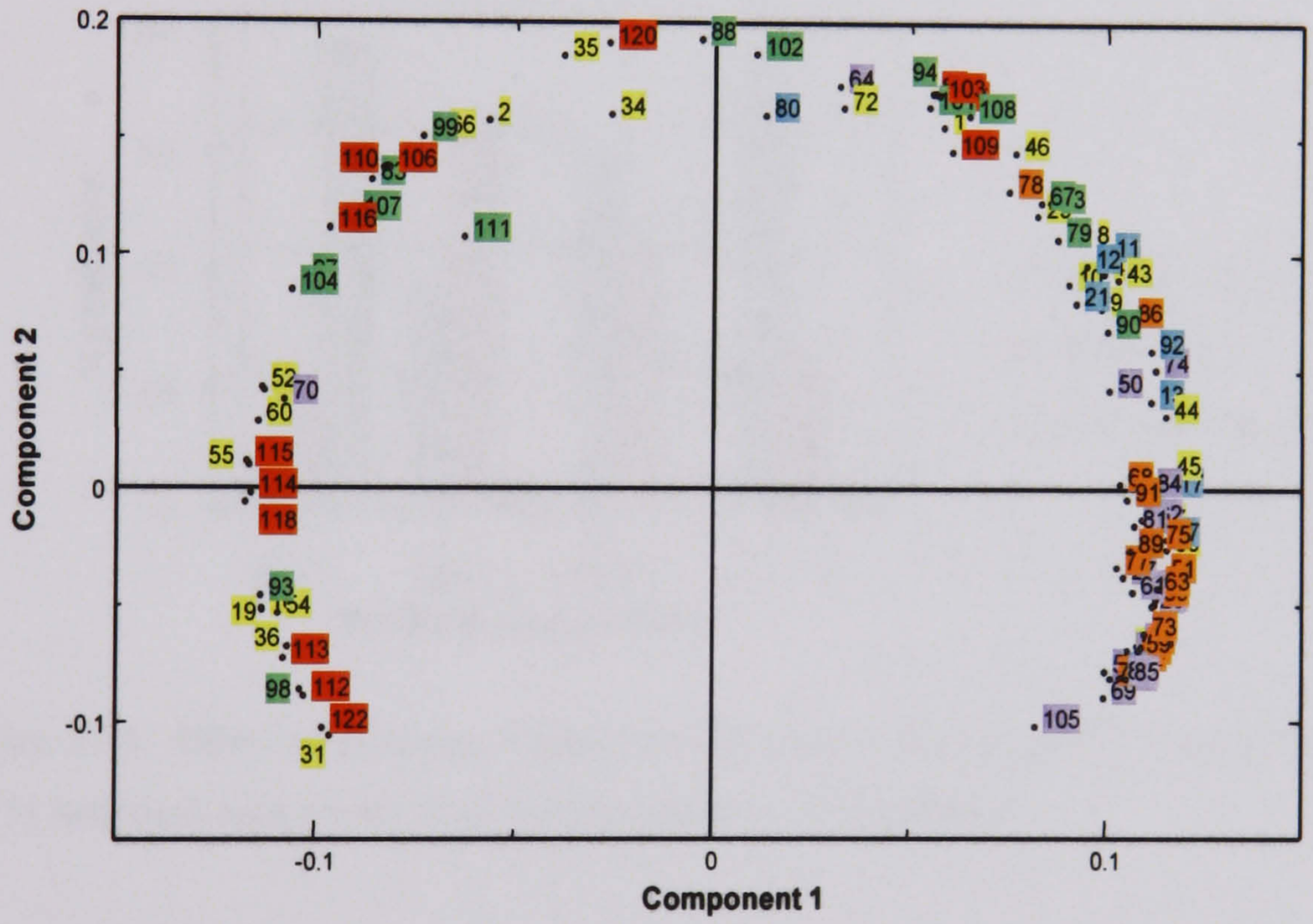
Table 2.7. ANOVA analysis of pyrolysis products produced at different temperatures – compounds showing no significant differences.

Peak number	Compound name	p value
6	Pentanedione	0.109
16	Furaldehyde	0.262
22	Acetolacetate	0.220
23	Methylfuranone 1	0.089
25	Cyclopentenedione 2	0.442
26	Cyclopentenedione 3	0.187
33	Hydroxycyclopentenone	0.991
38	Methylfuranone 3	0.163
42	Hydroxymethylcyclopentenone	0.097
65	Methoxymethylphenol	0.500
76	Propylphenol	0.088
92	Acetylphenol	0.242
100	Unknown guaiacyl isomer 2	0.053
119	Syringic acid	0.057
121	Ferulic acid	0.279
122	Dimethoxycinnamic acid	0.485

The remaining 106 compounds were re-normalised and the normalised peak area data are shown in Table 2.8 (Appendix B). These data were subsequently analysed using principal components analysis (PCA) (as described in Chapter 2.15.2). Using pyrolysis temperature as a factor, ANOVA was used to analyse principal component (PC) scores to determine which PCs showed significant differences between the different temperatures. In this way, PCs 1–3 were all found to significantly differentiate samples pyrolysed at different temperatures. However, the first two explained a total of 92.9% of variance and PC 3 explained only 3.8% of variance so was not considered of relative importance. The first two PCs are plotted in Fig. 2.12. In Fig. 2.12a there is clearly a trend on PC 1 where increasing temperature correlates with an increasing value for this PC. Also, PC 2 separates 358 °C and 770 °C from 610 °C and, in particular, 510 °C. Given that the first two PCs explained 92.9% of variance, it was generally possible to directly correlate the position of compounds on the loadings plot with their relative abundance at a particular temperature (Fig. 2.12b).



a



b

Figure 2.12. a: PCA analysis of peat pyrolysis at different temperatures. PCs 1 and 2 shown. b: Loadings plot for PCA analysis. Compounds are represented by their peak numbers as per Table 2.6 (Appendix A). Colour coding refers to compound classes defined in Table 2.6.

Phenolic compounds

At 358 °C, the most characteristic phenolic compounds were relatively large lignin-derived monomers. Most of these were syringyl compounds though two guaiacyl compounds were also present (vanillin (**93**) and an unknown guaiacyl monomer (**98**)). At 510 °C, the large lignin-derived compounds remained the most prominent phenolic compounds though the proportion of guaiacyl compounds had increased. By 610 °C, the lignin-derived ketones and aldehydes were less common whilst those lignin derivatives with alkyl side chains became relatively abundant. As the pyrolysis temperature tended towards 770 °C, the proportion of non specific phenols increased. To gain a quantitative perspective, the relative abundances of three compounds (coniferaldehyde (**117**), guaiacol (**53**) and *m*-/*p*-cresol (**51**)) were plotted (Fig. 2.13). This demonstrated how dramatically temperature can affect the relative abundances of pyrolysis products.

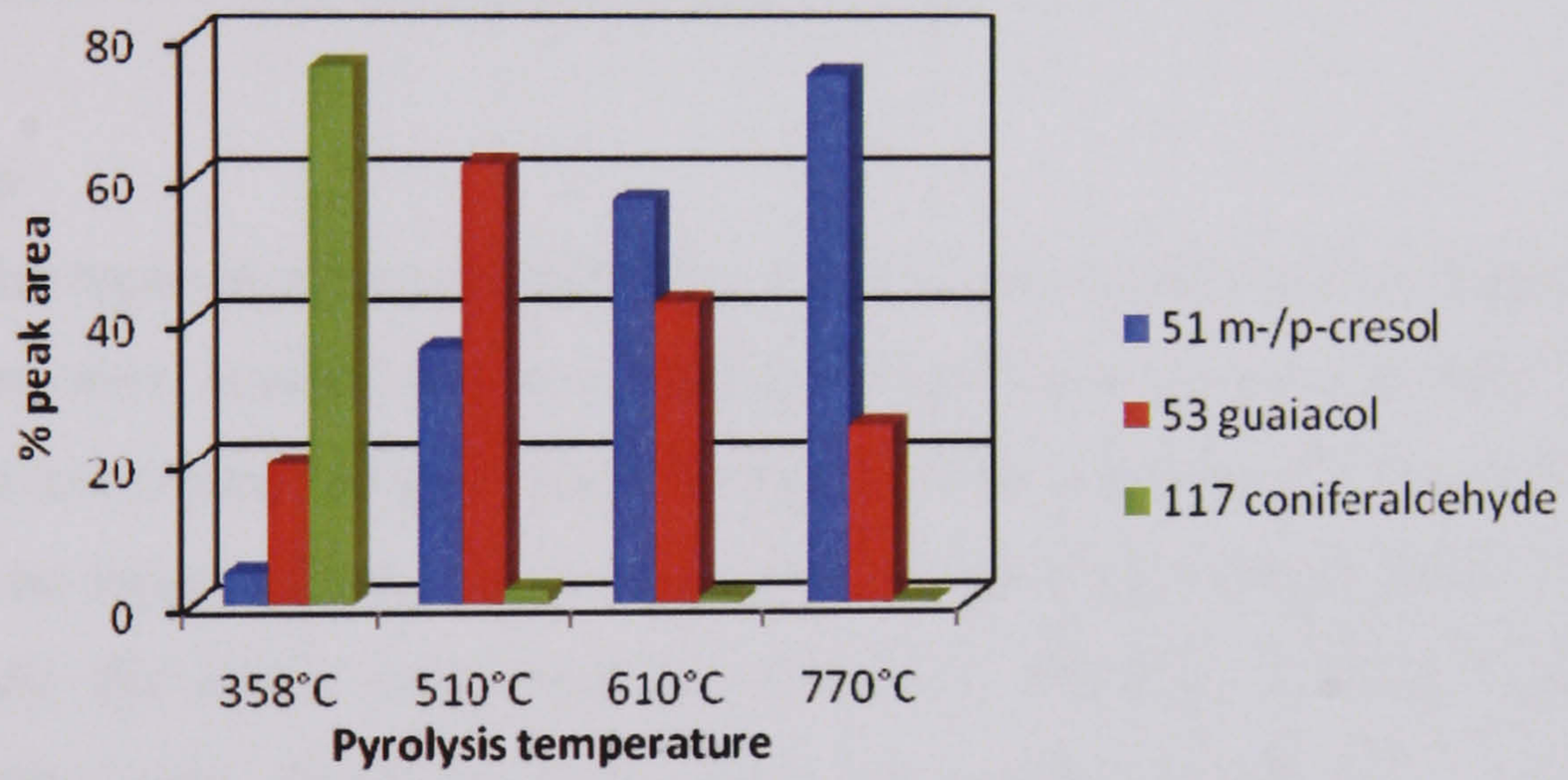


Figure 2.13. Effect of pyrolysis temperature on relative abundances of compounds. Data are % total peak area for the three compounds at each temperature.

Carbohydrate derivatives

As with the phenolic compounds, this group of compounds was distributed across the temperature range analysed. The pattern of this distribution was less clear than in the case of the phenolic compounds though at higher temperatures, simple alkyl furan molecules and cyclic ketones became more prominent.

Nitrogen-containing compounds

As the temperature tended towards 770 °C, the relative abundance of the nitrogen-containing compounds generally increased. One exception to this was indole (**80**) which was most abundant at 510 °C.

Aromatic compounds

This group included alkylbenzenes, naphthalenes, benzofurans and acetophenone. A similar pattern was found for the aromatic compounds as for the nitrogen-containing compounds with relative abundances increasing with temperature. Here again there were some exceptions to this pattern: benzoic acid (**64**) was most abundant at 510 °C whilst dihydrobenzofuran 2 (**70**) was most abundant at 358 °C.

Conclusions

Generally the types of molecules that were found to be characteristic of higher pyrolysis temperatures were smaller and less specific to particular parent materials than those generated at lower temperatures. This finding is similar to that found in a pyrolysis study carried out on wood and its components over the temperature range 400–1000 °C [96]. In that study, the lignin component of pine wood, which is composed primarily of guaiacyl units, was found to break down to simple phenols and also aromatic hydrocarbons at temperatures above 600 °C. This was most likely due to the higher temperatures increasing the possibility of secondary reactions that are responsible for the thermal breakdown of the larger compounds.

A pyrolysis temperature of 610 °C was used for further analyses as this provided a compromise between the high and low temperature products of pyrolysis. Also, this temperature had been used previously for the analysis of peat [45].

2.5 Determination of Phenols in Barley Malt Steam Distillates by High Performance Liquid Chromatography (HPLC)

2.5.1 Standards

Stock standard solutions of a range of phenols- phenol, *m*-cresol, *o*-cresol, *p*-cresol, 4-ethylphenol, guaiacol, 4-methylguaiacol, 4-ethylguaiacol - were prepared in acidified acetonitrile (1% (v/v) acetic acid/ acetonitrile) at a concentration of 400 mg L^{-1}. *m*- & *p*-Cresol were dissolved to give a combined concentration of 400 mg L^{-1}. These two compounds co-elute on the chromatographic system and were measured as the sum of the two components in the ratio of 1:1. Mixed calibration standards were made up in 1% (v/v) acetonitrile/ water in the range 0.01 to 0.5 mg L^{-1}. The internal standard, 2, 3, 5 trimethylphenol, was prepared in acidified acetonitrile at a concentration of 400 mg L^{-1}.

2.5.2 Steam distillation

Fifty one grams of malt was weighed into a milling beaker. The malt was milled using a Buhler Miag Universal Disc Mill (Buhler GmbH, Braunschweig, Germany, Post fach 3369, D-38023) which was set with a gap width of 0.2 mm using a feeler guage and the weight of the resulting grist was adjusted to 50 g ± 0.0005 g.

One hundred and fifty millilitres of UHQ water was preheated to 70 °C and added to the grist in a 500-mL round bottomed flask. The flask was place in a water bath at 65 °C for 1 hour, being shaken every 10 min. The flask was then left to cool and 10 mL 85% orthophosphoric acid was added. Steam distillation was then carried out at approximately 5 mL min^{-1} collecting 500 mL in a volumetric flask. All extracts were filtered directly into a vial through a syringe filter prior to HPLC analysis.

2.5.3 Analytical instrumentation

Analysis was carried out on Hewlett Packard 1050 HPLC system fitted with programmable fluorescence detector 1046A (Agilent Technologies UK Limited, Stockport, Cheshire, UK, SK8 3GR). Analytical column: Hypersil Excel ODS 5 µm, 250 mm x 4.6 mm (id) (Hichrom, Theale, Berkshire, UK, RG7 4PE). Guard column: Hypersil Excel ODS 5 µm, 50 mm x 4.6 mm (id) (Hichrom, Theale, Berkshire, UK, RG7 4PE). Column temperature: 30 °C (approx.). Injector programme: 80 µl extract/standard/control + 2 µl internal standard. Solvent system: water with 0.5% (v/v) acetic acid (A), acetonitrile with 0.5% (v/v) acetic acid (B) (Table 2.9).

Table 2.9. HPLC solvent gradient profile.

Step	Time (min)	A (%)	B (%)	Flow (mL min^{-1})
1	0	90	10	1
2	15	80	20	1
3	20	75	25	1
4	25	75	25	1.1
5	40	70	30	1.2
6	54	70	30	1.2
7	55	0	100	1.5
8	65	0	100	1.5
9	66	90	10	1.5
10	70	90	10	1

Detector conditions:

Excitation wavelength : 265 nm
Emission wavelength : 310 nm
Lamp power : 3 units (5w/220HZ)
Response time : 4000
Photomultiplier gain : 14 units

Concentrations were calculated using linear regression curves derived from calibration standard data. Final concentration values were expressed as the amount of phenol found in the initial malt barley sample, (mg kg^{-1}), taking into account the initial sample preparation procedure. For example, if 50 g of malt generated 500 mL of steam distillate then there will be a 10-times multiplication from mg L^{-1} in distillate to mg kg^{-1} in malt.

2.6 Headspace Solid Phase Micro Extraction (HS-SPME)-GC-MS Analysis of Marker Phenols in Peated Malt

2.6.1 Standards

Stock standard solutions of a range of phenols- phenol, *m*-cresol, *o*-cresol, *p*-cresol, 4-ethylphenol, guaiacol, 4-methylguaiacol and 4-ethylguaiacol - were prepared in ethanol solution. A stock standard solution of internal standard, 2, 3, 5-trimethylphenol, was also prepared in ethanol solution.

2.6.2 Sample preparation

The method development stages for this analysis are described in Chapter 2.6.4.

Five grams (± 0.5%) of malt sample was weighed directly into a 100-mL reagent bottle. Twenty five mL of 40% EtOH/UHQ water extraction solvent was added. The internal

standard was added at a concentration of 4 mg kg^{-1}. Samples were incubated in a Jeio Tech shaking incubator (Medline Scientific Ltd., Chalgrove, Oxon, UK, OX44 7RW) at 20 °C overnight whilst being constantly agitated at 100 rpm. One millilitre of the extraction solution was then diluted 1 in 10 and adjusted to 20% EtOH with UHQ water and 2 mL of this solution was sealed in a 10-mL headspace vial.

The method of standard additions was used for quantification. Standard additions were made to an unpeated malt, extracted using the same method as for peated malt, to create a 6 point calibration curve. The values for peated malts were calculated by interpolation on this curve. Mixed calibration standards were added in the ranges shown in Table 2.10.

Table 2.10. Calibration standard ranges for HS-SPME analysis of peated malt. Values are mg per kg of malt.

Compound	**Calibration range (mg kg^{-1})**
Guaiacol	0–35
Methylguaiacol	0–30
o-Cresol	0–18
Phenol	0–125
Ethylguaiacol	0–16
p-Cresol	0–48
m-Cresol	0–18
4-Ethylphenol	0–22

The SPME fibre used was an 85 µm polyacrylate (PA) (Supelco UK, Gillingham, Dorset, UK, SP8 4XT) as this fibre has been found previously to be useful for the analysis of phenols [104]. Sample pre-incubation time was 20 min at 50 °C. Extraction time was 15 min.

2.6.3 Analytical instrumentation

Analyses were performed with a Trace GC-MS (Thermo Fisher Scientific Inc., Waltham, MA 02454, USA) and used a 60 m x 0.32 mm DB-Waxetr capillary column with a film thickness of 1 μm (J & W Scientific, Stockport, Cheshire, SK8 3GR, UK). The carrier gas was He at a flow-rate of 1.4 mL min^{-1}. The initial oven temperature was 60 °C, held for 1 minute, increasing to 240 °C at 4 °C min^{-1} with a final hold time of 5 min. The SPME fibre was thermally desorbed in the programmed temperature vaporiser (PTV) injector. Initial temperature was 120 °C increasing to 300 °C at 3 °C sec^{-1} and held for 5 min. The split valve and septum purge were closed for 4 min. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans s^{-1}.

To diminish co-elution problems, all quantifications were made on integrated quantitation ion peaks. Compound identities were obtained by analysing reference samples of the pure compounds.

2.6.4 Method development

Initial analyses

A typical HS-SPME chromatogram from the analysis of 1 g of dry commercially produced peated malt shows that the eight most quantitatively significant phenols in peated malt, referred to as the marker phenols, could be detected in the malt headspace using this method (Fig. 2.14).

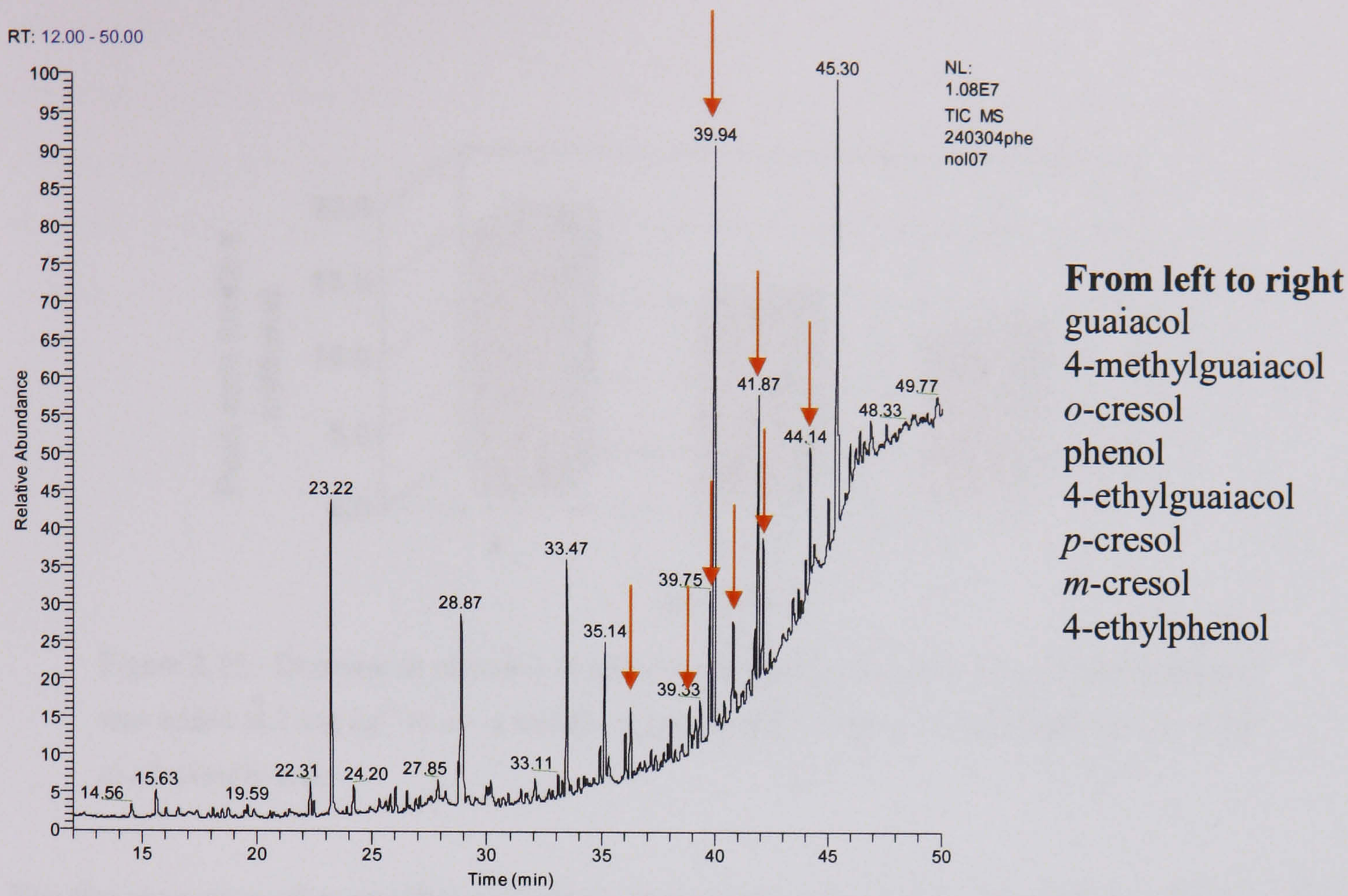


Figure 2.14. Typical HS-SPME trace from the analysis of dry peated malt (produced using Gartbreck peat).

If phenol standards were added to a dry malt sample, however, then the levels of these phenols were seen to decrease with time (Fig. 2.15). The standard used was 2, 3, 5 trimethylphenol.

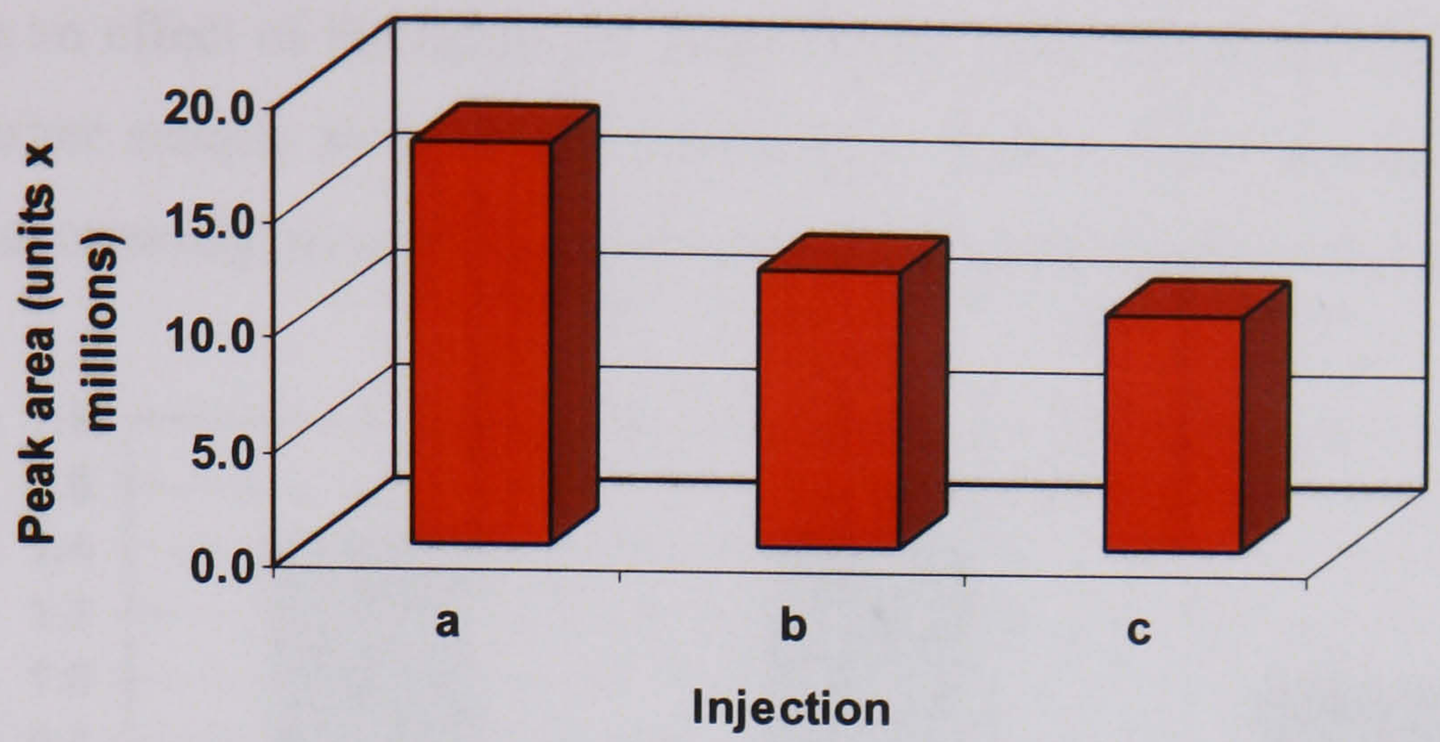


Figure 2.15. Decrease in response of added compounds over time. 2, 3, 5-trimethylphenol was added at 2 mg kg^{-1} to a 1 g sample of peated malt. Data are repeat injections (a, b and c) of a single vial.

For the purposes of quantifying phenols in peated malts, it was necessary to find a way of stabilising exogenous compounds prior to analysis. It was postulated that exogenous compounds required time to reach equilibrium between being bound to the malt and being unbound. Exogenous compounds were found to be stabilised by leaving samples in the headspace vials on the bench overnight prior to analysis (Fig. 2.16).

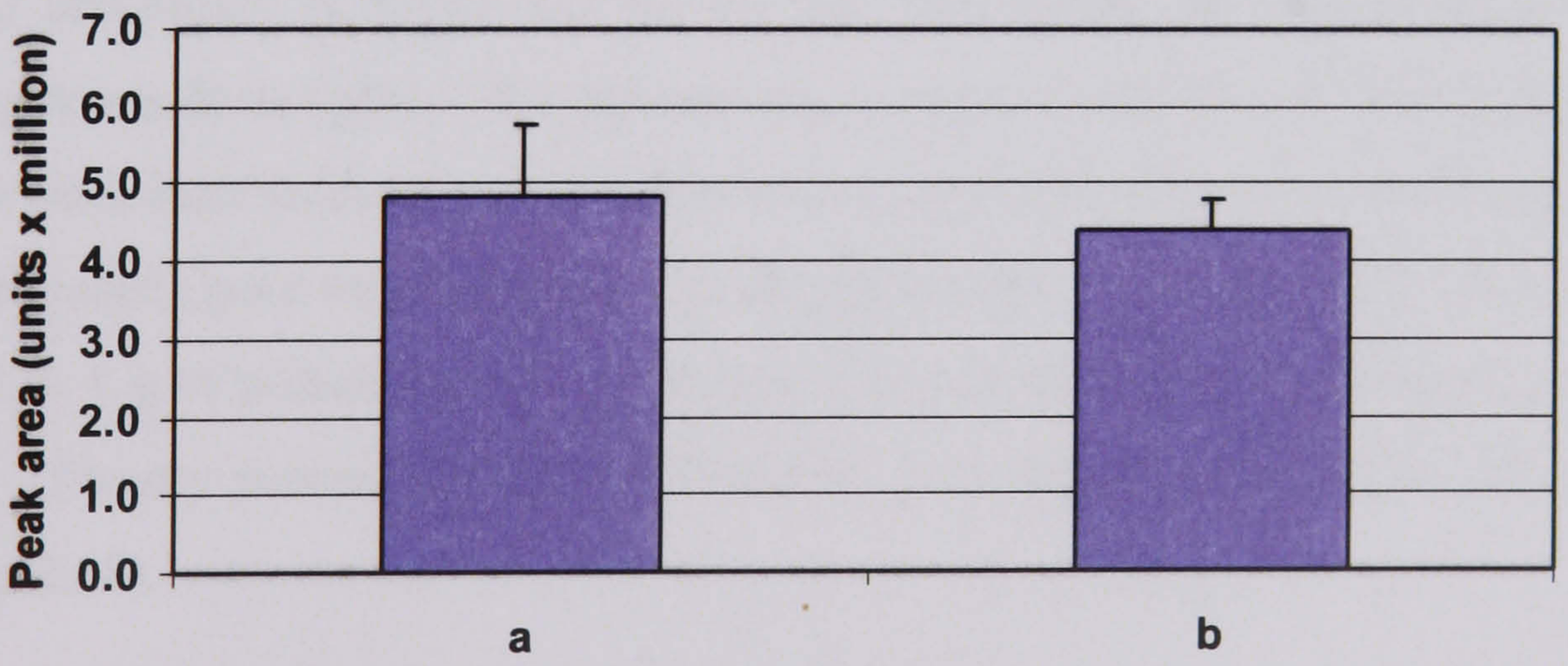


Figure 2.16. Stabilisation of added compounds by leaving vials overnight. 2, 3, 5-trimethylphenol was added at 2 mg kg^{-1} to 1 g of unpeated malt. Data are repeat injections (injections a and b) of three vials.

Whilst leaving samples overnight helped to stabilise exogenous compounds, it was noted that endogenous compounds did not give a reproducible response (Fig. 2.17). This was thought to be an effect of the relatively small sample size. Subsequently, it was decided that using larger sample sizes would reduce this effect. Given the limitations of the headspace vial capacity, an additional external extraction step would be required.

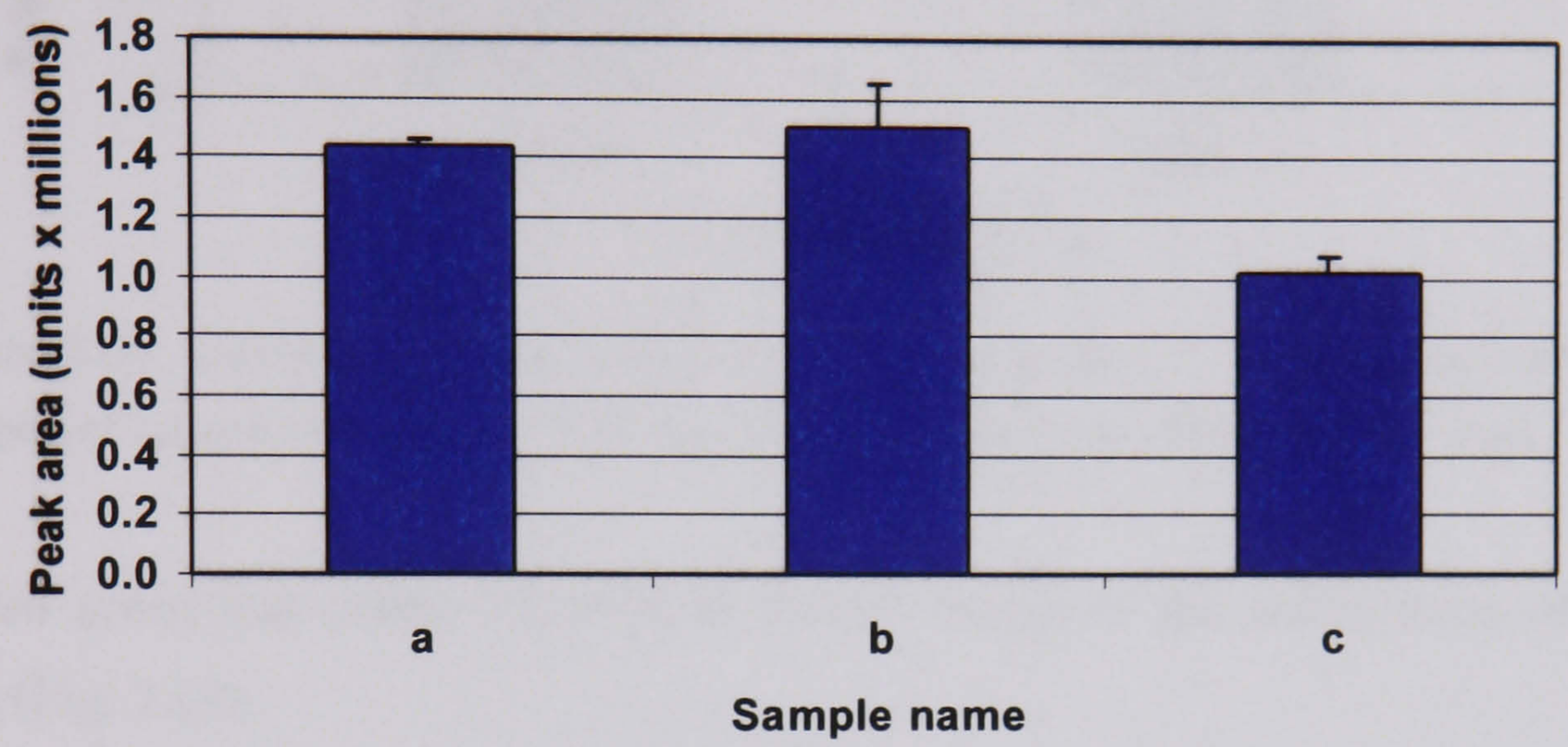


Figure 2.17. Effect of sample size on reproducibility. Three dry peated malt samples (a-c) of 1 g analysed in duplicate. Data are for guaiacol.

Prior to carrying out external extractions, it was necessary to determine the most appropriate solvent for the optimum adsorption of the phenols to the SPME fibre. Work carried out previously at SWRI had shown that 20% EtOH gave good results for the release of phenols from spirit. This solvent was compared with 40% EtOH for the release of phenols from malt extracts (UHQ water was discounted as this was previously found to give particularly poor reproducibility for alkylguaiacols). In each case 2 mL of solvent was added to 1 g of peated malt in headspace vials and the vials were left to equilibrate overnight. Twenty percent EtOH was found to give higher responses for all phenols tested (Fig. 2.18).

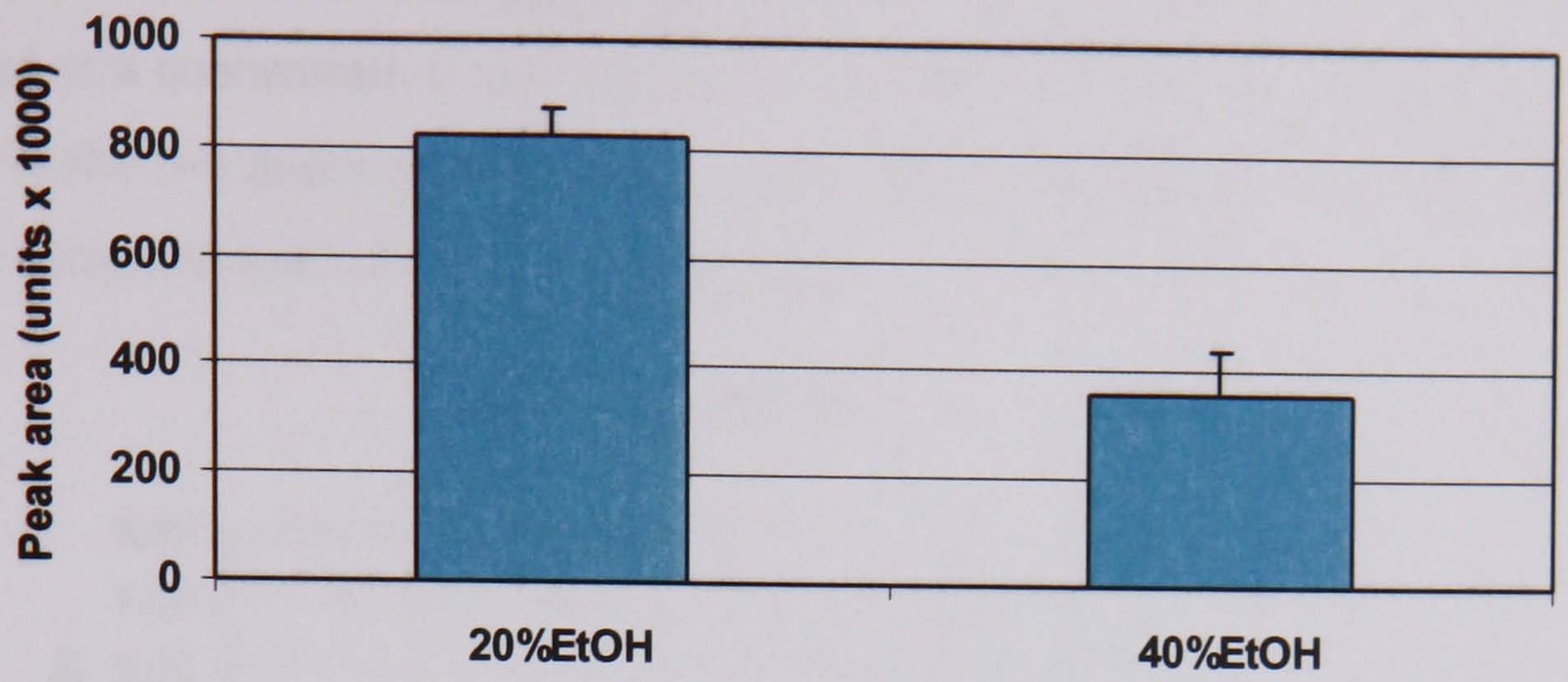


Figure 2.18. Comparison of two solvents for HS-SPME analysis. Three peated malt samples of 1 g analysed in duplicate for each ethanol concentration. Data are for *o*-cresol.

It was also noted that added 20% EtOH further improved the stabilisation of added standards (Fig. 2.19).

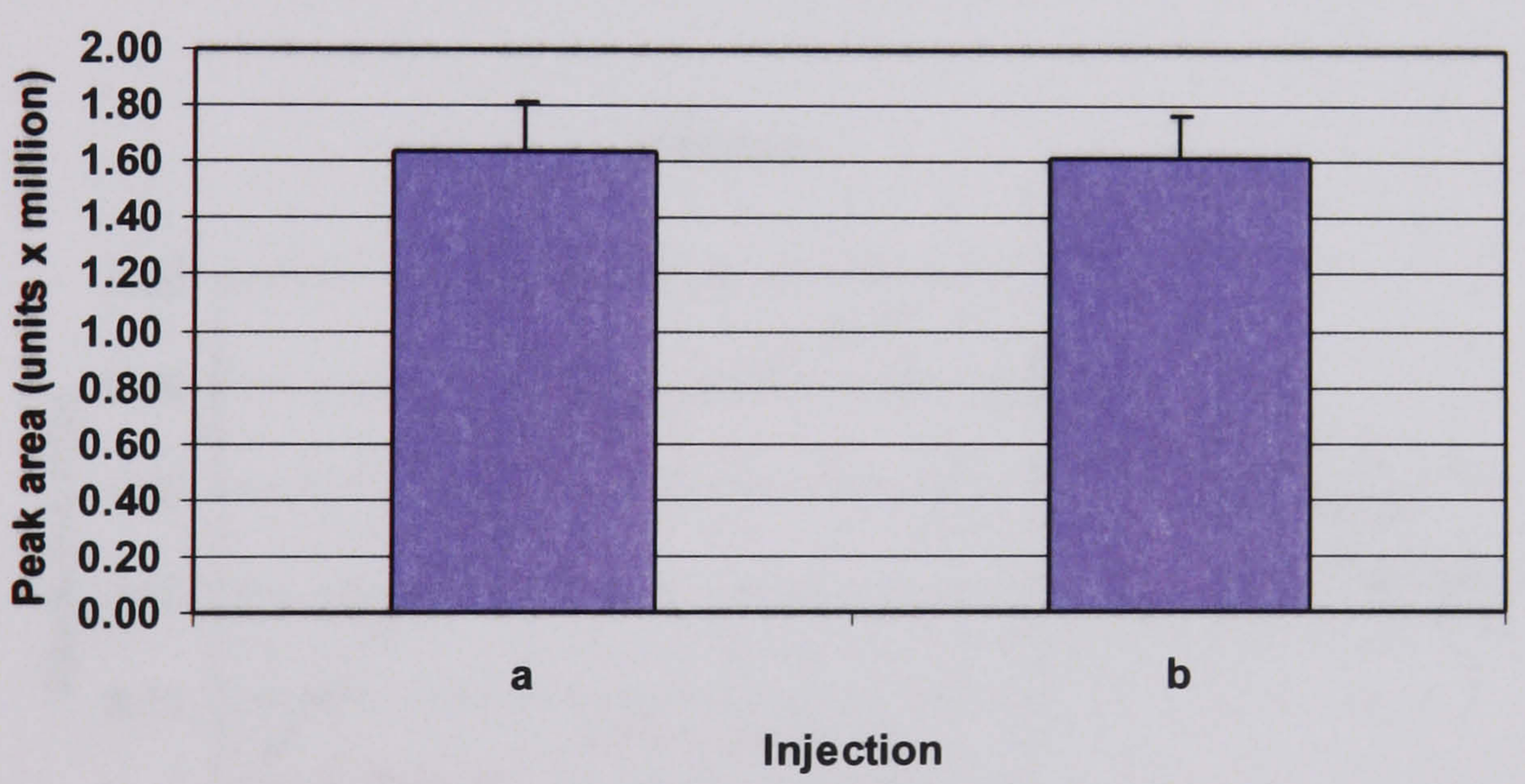


Figure 2.19. Stabilisation of added compounds by leaving vials overnight. Data are for 2, 3, 5-trimethylphenol which was added at 2 mg kg^{-1} to 1 g of unpeated malt + 2 mL 20% EtOH. Data are duplicate injections (a and b) of three vials.

To test whether the method of standard additions would have to be employed to quantify phenols in peated malt, calibration curves were prepared in 2 mL 20% EtOH and also in 2 mL 20% EtOH in the presence of 1 g of unpeated malt. For this analysis, peak areas were reported as response ratios where the ratio of compound peak area is calculated

relative to the internal standard peak area. The internal standard (2, 3, 5-trimethylphenol) was added at a concentration of 2 mg kg^{-1}. The gradients of the calibration curves were different in the two matrices (Fig. 2.20). Therefore quantification would be most reliably achieved using the method of standard additions.

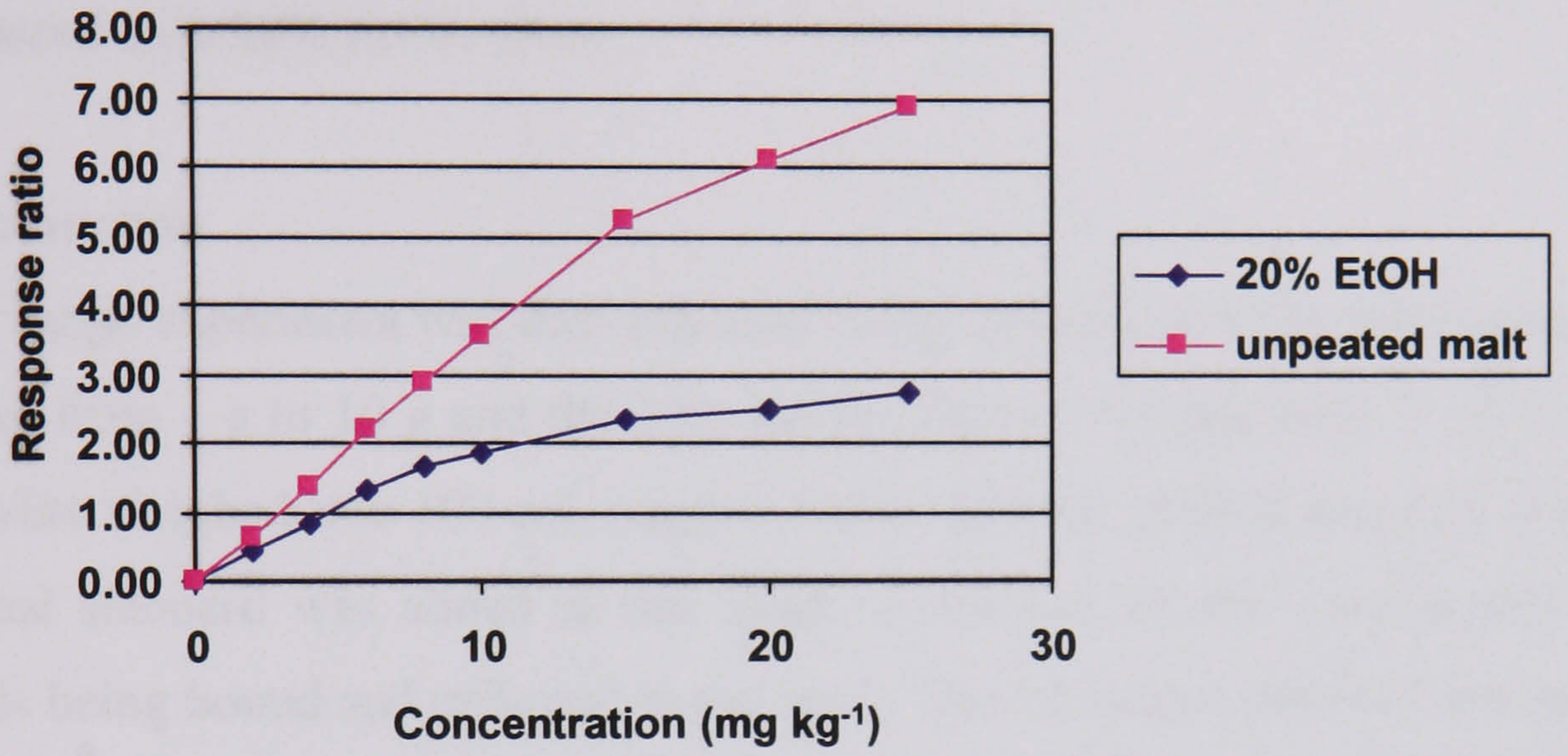


Figure 2.20. Calibration curves obtained using 20% EtOH or 20% EtOH in the presence of 1 g of unpeated malt. a: calibration curves for *o*-cresol. b: calibration curves for *p*-cresol.

These data were also used to estimate the linear range maximums of the HS-SPME method. Inspecting the calibration curves by eye, the linear range maximum was between 8 and10 mg kg^{-1} in the absence of malt for all phenols. In the presence of malt the linear range maximum was approximately 15 mg kg^{-1} for all phenols except *p*-cresol and 4-ethylphenol which both gave values of less than 10 mg kg^{-1}. This was because the recovery of *p*-cresol and 4-ethylphenol was found to be relatively poor in the presence of malt compared with 20% EtOH alone.

External extraction

The linear range experiment was then repeated using an external extraction increasing the sample size from 1 g to 10 g and the 20% EtOH solution volume from 2 mL to 20 mL. Samples were weighed into 100-mL reagent bottles and the internal standard was added. The internal standard was added at this stage to account for the equilibrium between compounds being bound and unbound to the malt. The extraction solution was added and samples were left overnight in an incubator set to 20 °C whilst being constantly agitated at 100 rpm. Two millilitres was taken from each extraction solution and added to headspace vials so as to analyse the samples at 20% EtOH. It was noted that the linear ranges of both *p*-cresol and 4-ethylphenol were now brought in line with the other compounds.

Ratio of malt to extraction solvent

The ratio of malt to extraction solvent was varied to see if the extraction volume used was limiting the recovery of the phenols. The extraction solution diluted 1 in 5 post extraction was compared with an extraction solution diluted 1 in 5 prior to extraction. This showed that diluting the extraction solution prior to extraction improved recoveries. This was especially apparent for *p*-cresol and 4-ethylphenol (Fig. 2.21).

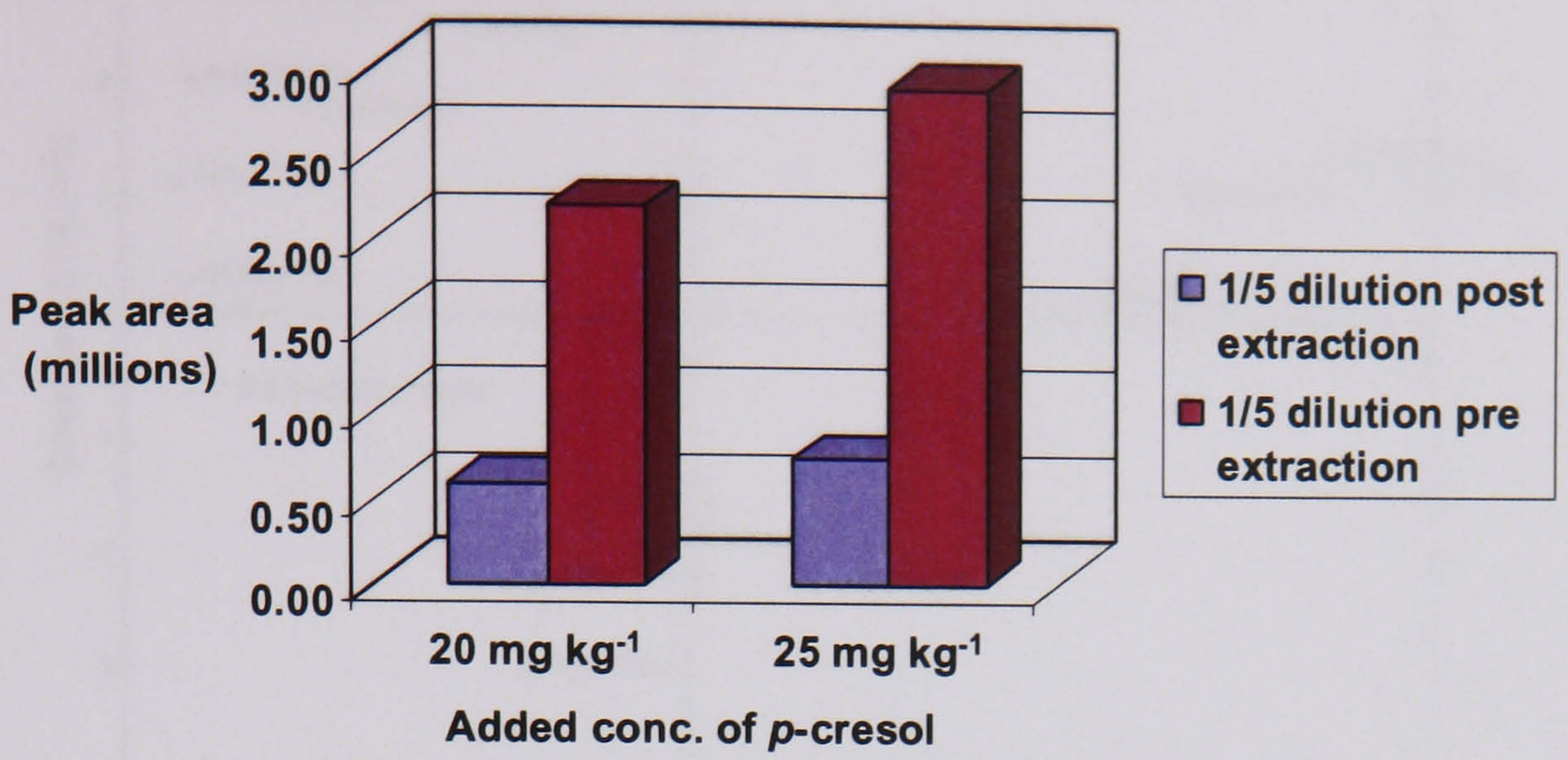


Figure 2.21. Effect of pre and post extraction dilution on recovery of *p*-cresol.

EtOH concentrations

As a range of different phenols with differing polarities were being analysed, the concentration of the EtOH extraction solution was varied to determine which gave the best extraction. Therefore, the external extraction procedure was carried out (without any dilutions pre or post extraction dilutions) on a peated malt using EtOH solutions in a range from 20% to 60%. Subsequent to the external extraction procedure, 2 mL of each extract was added to a headspace vial. The 20% solution was analysed as it was whilst the 40% and 60% solutions were diluted to 20% with UHQ water. The results were analysed by PCA (Fig. 2.22). These results show that different phenols were optimally extracted at different EtOH concentrations. Most of the marker phenols were optimally extracted at 20% EtOH. However, para substituted alkyl phenols (*p*-cresol and 4-ethylphenol) were better extracted at higher EtOH concentrations. Also, the internal standard was optimally extracted at 40% EtOH. It was therefore decided that for the analysis of phenols in peated malts, a 40% solution would provide a good compromise.

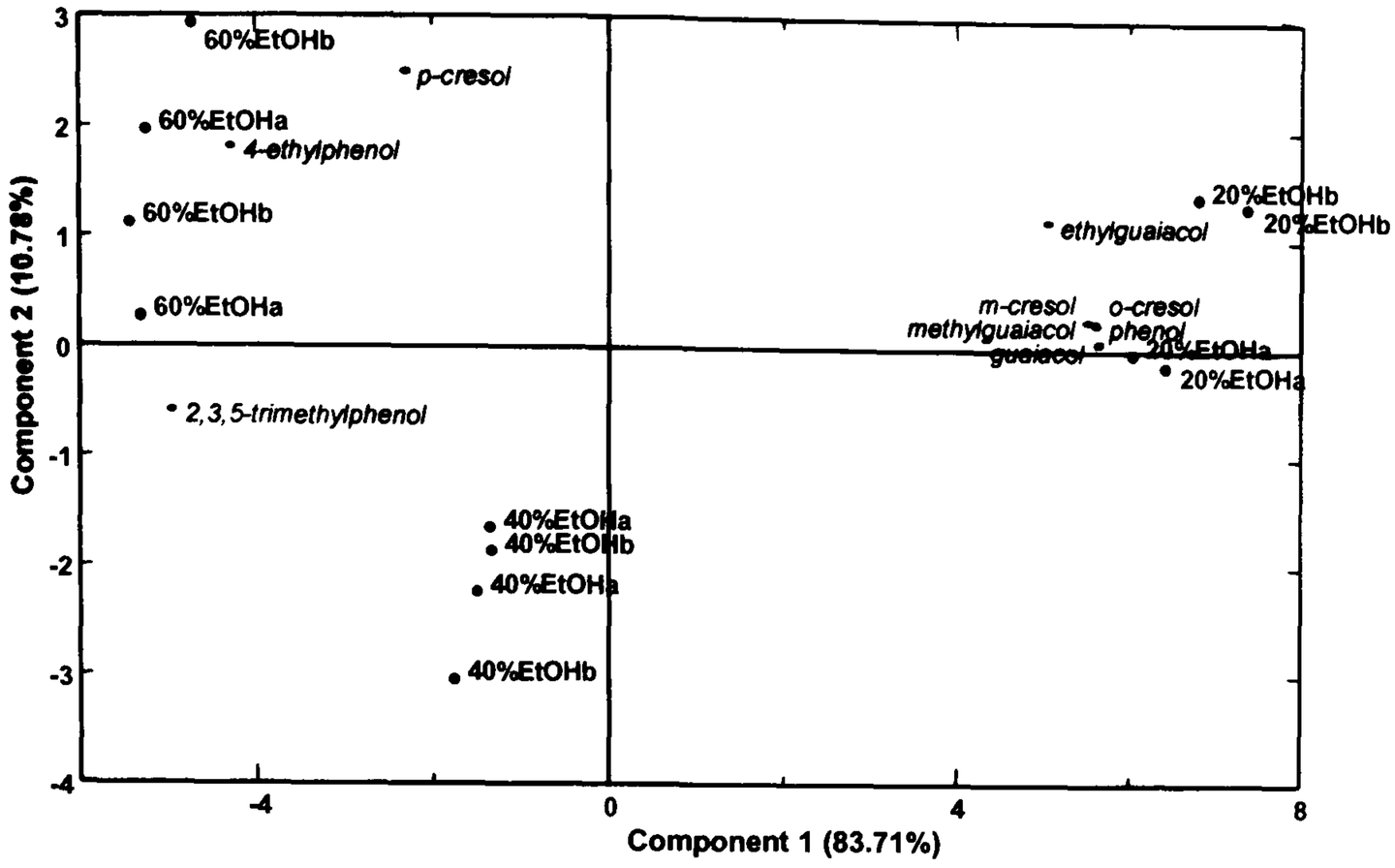


Figure 2.22. PCA of peak areas of the marker phenols in various concentrations of EtOH aqueous solution. Duplicate aliquots (a and b) from each extract were analysed and each replicate was injected twice.

Peated malt matrix effects

It was important to test whether standard additions could be made to an unpeated malt in order to produce one standard addition curve which could then be used for the calculation of phenol concentrations in peated malts. Standard additions were therefore made to an unpeated malt, a lightly peated malt and a heavily peated malt. Standards were added in a range that would cover typical commercially produced peated malts (Table 2.11).

Table 2.11. Top standards used for calibration curves.

Compound	**Top standard**
Guaiacol	5.3
Methylguaiacol	5.9
o-Cresol	9.4
Phenol	59.5
Ethylguaiacol	5.0
p-Cresol	19.4
m-Cresol	10.7
4-Ethylphenol	15.3

For this experiment, 5 g of peated or unpeated malt sample was weighed directly into 100-mL reagent bottles. Taking account of previous experimental data, a 40% EtOH solution was used as the extraction solvent (Fig. 2.22). Twenty five millilitres of extraction solvent was added to give a high ratio of solvent to malt as this was found to improve recovery of *p*-cresol and 4-ethylphenol (Fig. 2.21). Samples were incubated at 20 °C overnight whilst being constantly agitated at 100 rpm. One millilitre of the extraction solution was then added to a headspace vial and adjusted to 20% EtOH with UHQ water or the extraction solution was diluted 1 in 10 and adjusted to 20% EtOH and 2 mL of this solution was added to a headspace vial. The results of this experiment showed that using this range of standards, dilution was necessary for phenol. This was determined by examining the linearity of diluted and undiluted calibration curves (Fig. 4.23).

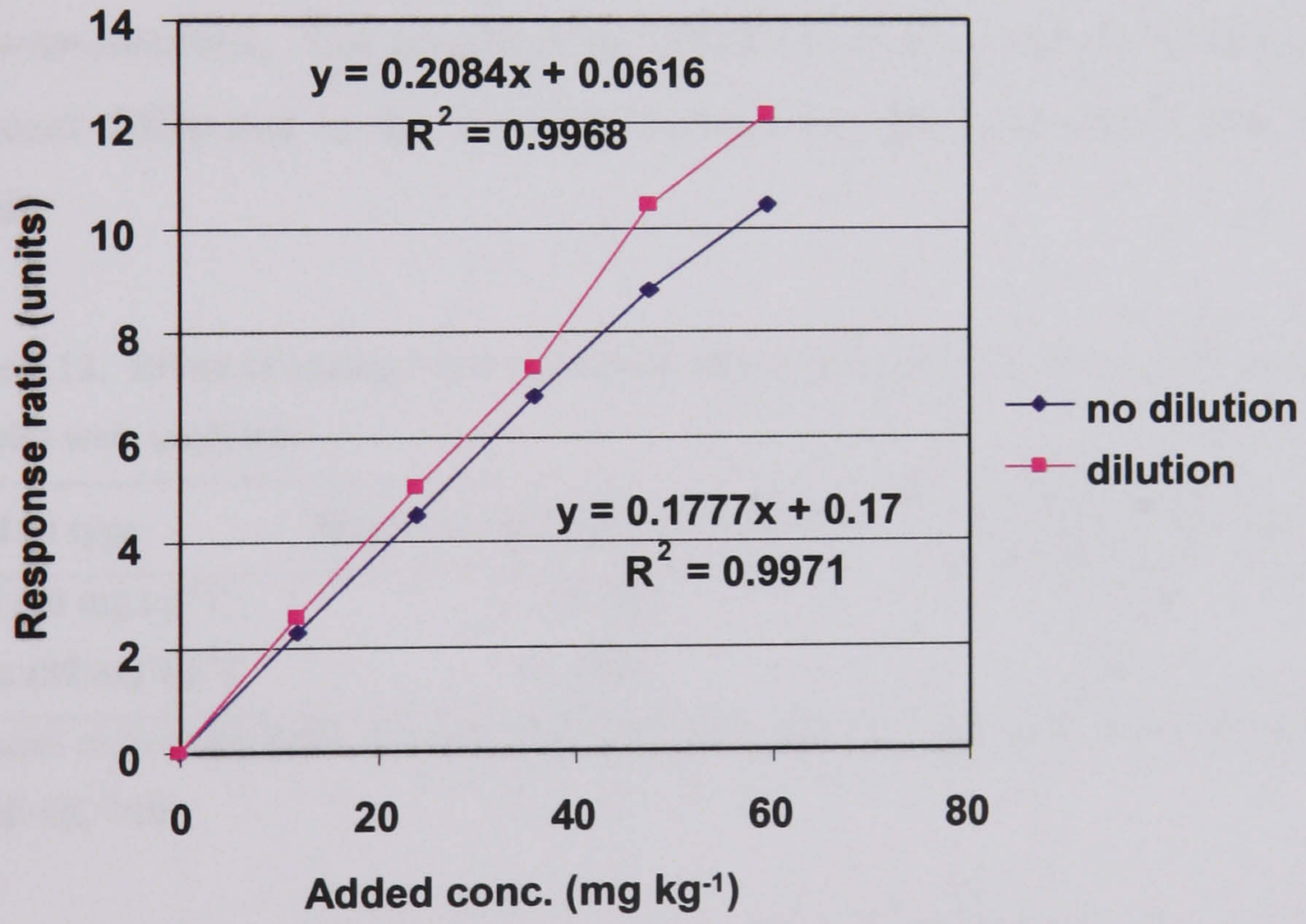


Figure 2.23. Calibration curves for phenol using undiluted and diluted extracts.

Therefore, considering only the diluted samples, the gradient of the curve produced using an unpeated malt was in good agreement with those produced using the peated malts (Table 2.12). This indicated that added compounds acted in a similar way when added to

a peated or to an unpeated malt. Therefore, standard additions could be made to an unpeated malt and the responses for peated malts could be interpolated onto this curve.

Table 2.12. Comparison of calibration curves using peated and unpeated malts. Data are for 4-ethylphenol.

Malt type	R^2	gradient	lower 95%	upper 95%
low peat (20 mg kg^{-1})[a]	0.9960	0.3990	0.3638	0.4343
high peat (82 mg kg^{-1})[a]	0.9987	0.4493	0.4268	0.4718
unpeated malt	0.9948	0.4192	0.3771	0.4613

[a] Figures in brackets refer to total marker phenols levels in the malts measured using HS-SPME-GC-MS.

It was also noted that using the HS-SPME method, which was more sensitive than the current HPLC method, a low level of endogenous 2, 3, 5-trimethylphenol could be detected in peated malt. Therefore, the effect of peating level on the internal standard response was observed. The results of an ANOVA (Table 2.13) showed that there was no significant difference in the level of internal standard recovered from low or high peated malt.

Table 2.13. Effect of peating level on internal standard response. For each malt type, five samples were analysed.

Malt type	Mean internal standard peak area	Standard Deviation	p value
low peat (20 mg kg^{-1})[a]	202699	10225	0.22
high peat (82 mg kg^{-1})[a]	210147	6991	

[a] Figures in brackets refer to total marker phenols levels in the malts measured using HS-SPME-GC-MS.

Nevertheless, for subsequent analyses, the level of internal standard was increased from 2 mg kg^{-1} to 4 mg kg^{-1} to minimise any effect of endogenous 2, 3, 5-trimethylphenol. Fig. 2.24 shows that during subsequent analysis of a range of peated malts from different commercial producers exposed to a wide range of peating levels, 2, 3, 5-trimethylphenol peak area was found to be unrelated to peating level. Also shown in Fig. 2.24 is the mean 2, 3, 5-trimethylphenol peak area from the six analyses of an unpeated malt, which was not significantly different from the levels found in the peated malts ($p = 0.237$).

Therefore the presence of endogenous 2, 3, 5-trimethylphenol did not appear to be significantly affecting the internal standard level.

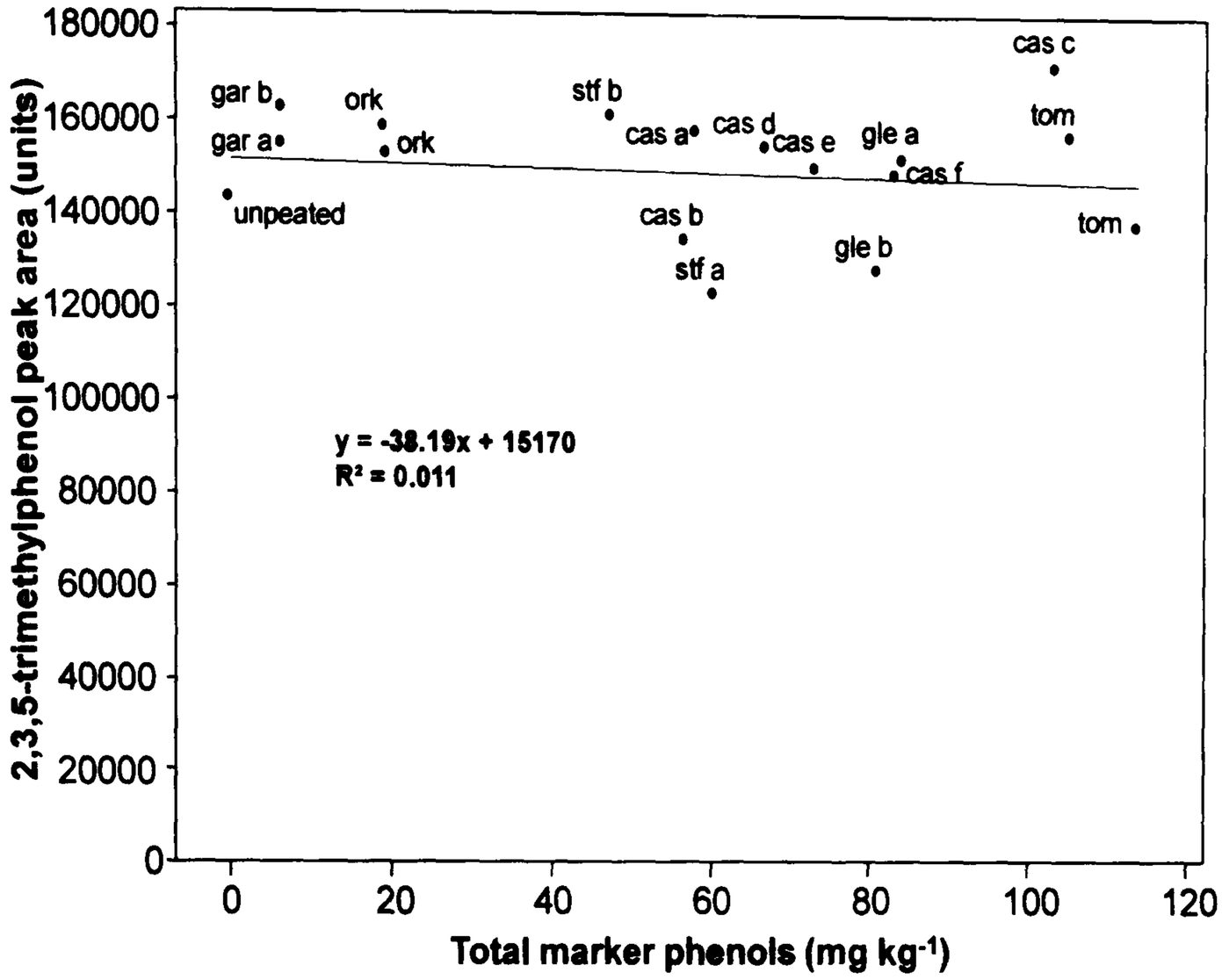


Figure 2.24. Relationship between 2, 3, 5-trimethylphenol response and peating level. Data are average of two injections. Added 2, 3, 5-trimethylphenol concentration: 4 mg kg^{-1}.

Limits of detection (LOD)

The limit of detection was determined by repeated analysis of a blank sample (unpeated malt) and using the equation:

$$y - y_B = 3s_B \quad (1)$$

Where y is the instrument signal corresponding to the limit of detection, y_B is the blank signal and s_B is the standard deviation of the blank signal. This limit could then be converted to a mg kg^{-1} value by using the linear regression curve. The estimated limits of detection are listed in Table 2.14.

Table 2.14. Limits of detection for HS-SPME method.

Compound	**LOD (mg kg^{-1})**
Guaiacol	0.36
Methylguaiacol	0.85
o-Cresol	0.06
Phenol	0.78
Ethylguaiacol	0.16
p-Cresol	0.15
m-Cresol	0.10
4-Ethylphenol	0.15

The limits of detection were calculated using the average of four linear regression curves. The value for methylguaiacol was negatively affected by variation in the regression curves for these compounds over this time period. Nevertheless, for the analysis of the majority of peated malts these estimated limits were adequate.

Comparison with steam distillation

Once a method had been developed for phenols analysis using HS-SPME, the accuracy of the method was tested by a comparison with the current method: steam distillation of malt followed by HPLC of the distillate. A survey of several commercially produced malts, from various producers, which had been subjected to varying degrees of peating, were analysed by both methods and the results compared where the current HPLC method was used as the reference method (Fig. 2.25 and Table 2.15). The relationship between HS-SPME and HPLC results was generally linear though this was less true for ethylguaiacol. However, HS-SPME tended to underestimate phenolic compound content. As can be seen from the gradient data in Table 2.15, this was truer for guaiacyl compounds than for other compounds.

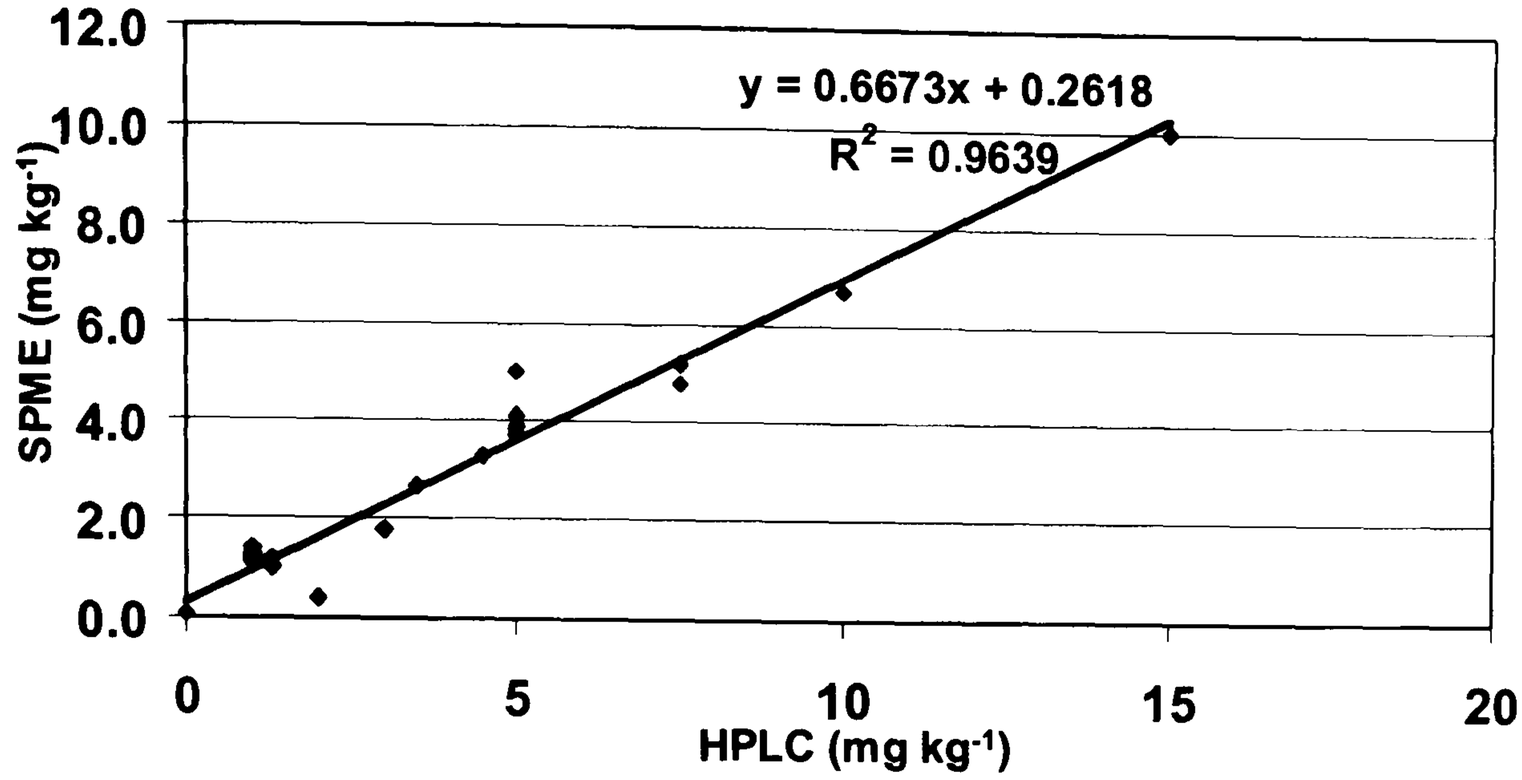


Figure 2.25. Comparison of concentrations of *o*-cresol in a range of peated malts as determined by HPLC and HS-SPME.

Table 2.15. Regression data for comparison of concentrations of marker phenols as determined by HPLC and HS-SPME.

Compound	Intercept	Gradient	Correlation Coefficient
Guaiacol	0.23	0.39	0.9424
Methylguaiacol	0.01	0.51	0.9199
o-Cresol	0.26	0.67	0.9818
Phenol	1.38	0.64	0.9824
Ethylguaiacol	0.13	0.25	0.7871
m-/*p*-Cresol	1.99	0.70	0.9398
4-Ethylphenol	0.27	0.55	0.9778

To assess the effect of the extraction procedure on the results, steam distillates from a selection of malts were analysed using the HS-SPME method. In this case, calibration standards were made up in 20% EtOH in headspace vials without any prior extraction. The results of using HS-SPME to analyse the phenolic content of steam distillates were plotted against those produced using HPLC (Fig. 2.26 and Table 2.16). When steam distillates were analysed using HS-SPME they gave a higher gradient against the HPLC method than when ethanol extracts were analysed. However, even when analysing steam

distillates, the HS-SPME results were still proportionally lower than those obtained using HPLC.

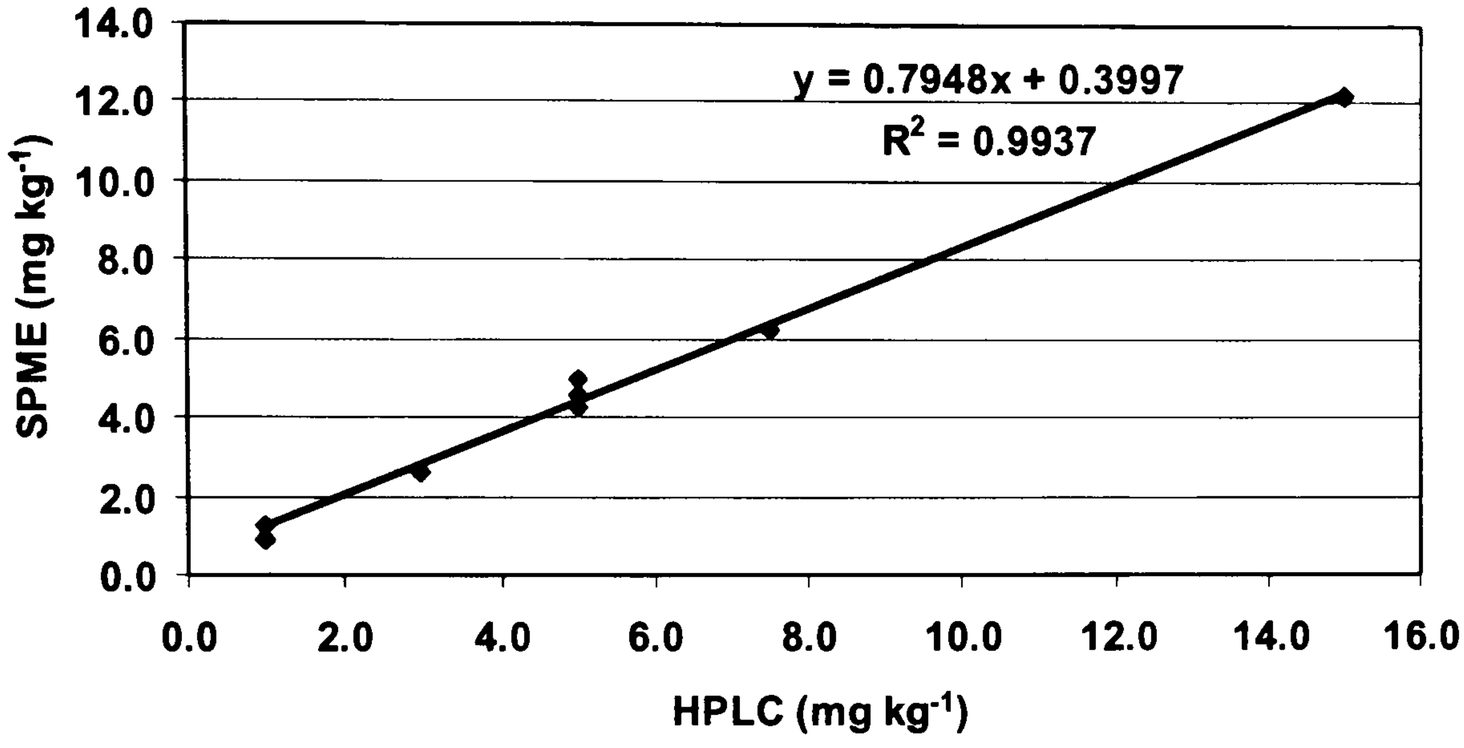


Figure 2.26. Comparison of concentrations of *o*-cresol in a range of peated malts as determined by HPLC and HS-SPME of steam distillates.

Table 2.16. Regression data for comparison of concentrations of marker phenols as determined by HPLC and HS-SPME of steam distillates.

Compound	Intercept	Gradient	Correlation Coefficient
Guaiacol	0.43	0.67	0.9566
Methylguaiacol	-0.09	0.68	0.8937
o-Cresol	0.40	0.79	0.9968
Phenol	3.48	0.73	0.9977
Ethylguaiacol	-0.02	0.41	0.9564
m-/*p*-Cresol	1.24	0.75	0.9980
4-Ethylphenol	0.30	0.54	0.9896

Analysis of laboratory scale samples

Preliminary studies showed that the levels of marker phenols in laboratory-produced samples were relatively high compared with commercially produced samples. Therefore,

the maximum range of the calibration curves was increased (Table 2.17). This was found to have no impact on the linearity of the curves (Fig. 2.27).

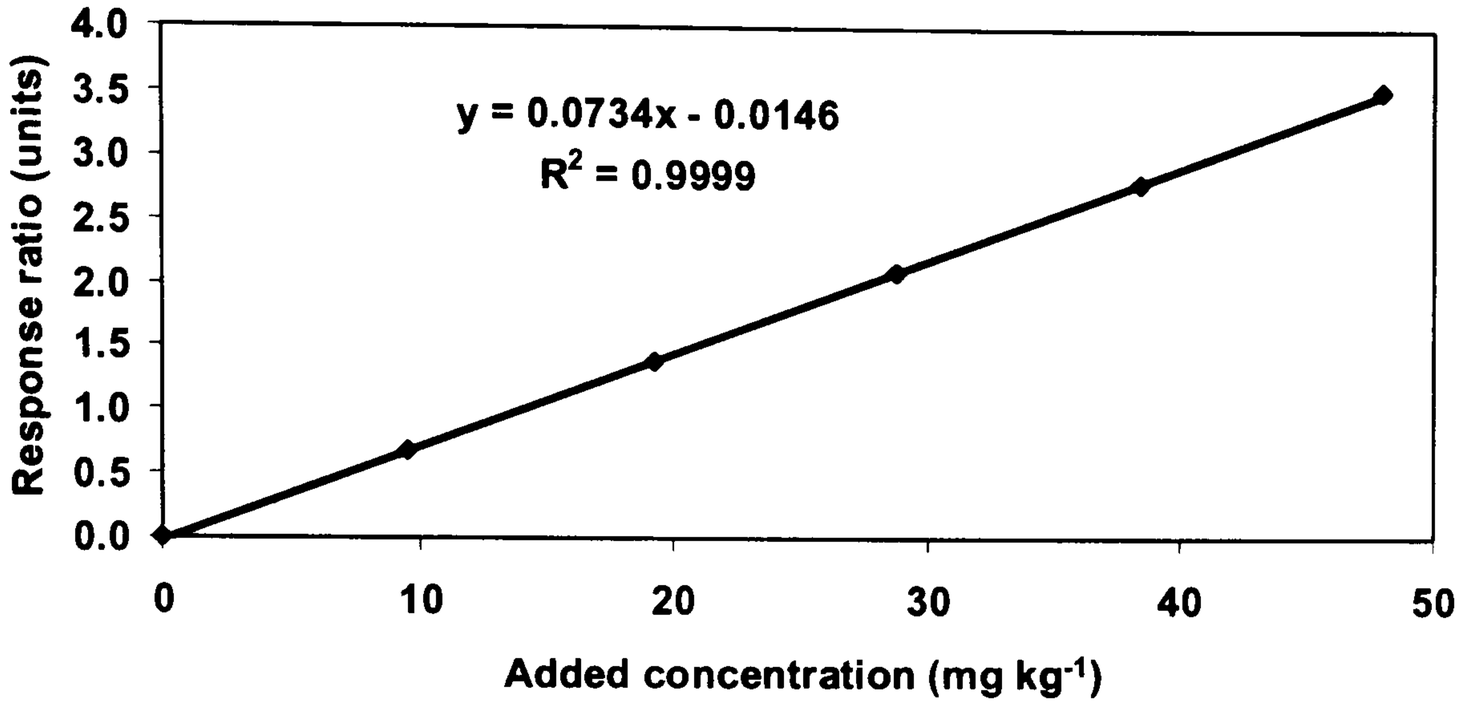


Figure 2.27. Calibration curve for *p*-cresol required for analysis of higly peated lab-scale peated malts.

Table 2.17. Comparison of calibration standards used for method development and those used for analysis of lab-scale samples.

Compound	Method development top standard	Lab-scale top standard
Guaiacol	5.3	34.8
Methylguaiacol	5.9	29.8
o-Cresol	9.4	17.8
Phenol	59.5	125.0
Ethylguaiacol	5.0	16.2
p-Cresol	19.4	48.0
m-Cresol	10.7	18.2
4-Ethylphenol	15.3	21.6

As it had been noted previously that some endogenous 2, 3, 5-trimethylphenol was detectable in peated malt, it was important to determine if the high peating levels found in lab-scale peated malts had a significant effect on the internal standard levels in these peated malts. Therefore, four peated malt samples produced using each of six peat types, were spiked with 4 mg kg^{-1} of 2, 3, 5-trimethylphenol and were analysed using the HS-

SPME method. The resulting 2, 3, 5-trimethylphenol peak areas were compared (Table 2.18). These data showed no significant effect of peat source on 2, 3, 5-trimethylphenol peak area. Therefore, within the lab-scale peated malt samples, endogenous 2, 3, 5-trimethylphenol did not significantly affect the internal standard level. However, there was a significant difference in 2, 3, 5-trimethylphenol peak area between unpeated and peated malt which was not found in the case of the commercial peated malts. Therefore, it may be that compounds quantified relative to the 2, 3, 5-trimethylphenol internal standard are underestimated in the case of lab-scale samples.

Table 2.18. Relationship between 2, 3, 5-trimethylphenol response and peat source. Mean values are the average peak areas of two injections of four samples. Added 2, 3, 5-trimethylphenol concentration: 4 mg kg^{-1}.

Peat source	Cases	Mean	Lower 95%	Upper 95%
Castlehill	8	182498.31	166540.37	198456.25
Gartbreck	8	187837.65	176955.23	198720.06
Glenmachrie	8	190966.14	182079.48	199852.80
Hobbister	8	183975.50	168217.77	199733.22
St Fergus	8	195806.93	186333.24	205280.62
Tomintoul	8	182790.25	168809.37	196771.13
Unpeated	8	153831.15	144304.75	163357.55

Conclusions

HS-SPME in conjunction with GC-MS was found to provide a faster, more convenient method than the existing HPLC method for the quantification of marker phenols in malt. The fact that the ethanol extraction method used for the HS-SPME analysis did not yield values as high as when the steam distillates were analysed using HS-SPME may reflect the relatively gentle nature of the ethanol extraction. The steam distillation may recover more phenols from malt samples than the simple ethanol extraction. HS-SPME analysis of either the ethanol extracts or the steam distillates did not give results as high as when HPLC was used. There was however, a linear relationship between the HS-SPME results and the HPLC results. Therefore, the fact that the HS-SPME method gave proportionally different results to the HPLC method was not of great importance for this particular study

as the main point of interest was the relative differences between different malts. Further work would be needed to improve the accuracy of the HS-SPME method.

2.7 Solid-Phase Extraction (SPE)-GC-MS Analysis of Peated Malt

2.7.1 Standards

Stock standard solutions of the internal standards - 2, 3, 5-trimethylphenol, naphthalene-d8 and pyridine-d5 - were prepared in ethanol solution.

2.7.2 Sample preparation

Five grams (± 0.5%) of each malt sample to be analysed was weighed into 100-mL reagent bottles and extracted in 40% EtOH solution as described for HS-SPME method (Chapter 2.5.2). Internal standards were added to malt at the following concentrations: 2, 3, 5-trimethylphenol, 4 mg kg^{-1}; naphthalene-d8, 0.2 mg kg^{-1} and pyridine-d5, 1 mg kg^{-1}. As for the HS-SPME method, these internal standards were added to the malt samples prior to extraction to account for the equilibrium between being bound or unbound to the malt. Compounds were quantified relative to an internal standard: pyridine-d5 for nitrogen-containing compounds, 2, 3, 5-trimethylphenol for phenolic and carbohydrate-derived compounds and naphthalene-d8 for other aromatic compounds.

Ten millilitres of extraction solution was diluted to 10% EtOH with 30 mL of UHQ water. Phenomenex Strata-X SPE cartridges (with a sorbent mass of 200 mg and reservoir volume of 3 mL) (Phenomenex, Macclesfield, Cheshire, UK, SK10 2BN) were used in accordance with the manufacturer's instructions in order to extract and concentrate the peat-derived compounds from the ethanol extracts. The SPE cartridges were conditioned prior to use. Cartridges were conditioned firstly with DCM using approximately three times the volume of the cartridges. Cartridges were then attached to vacuum apparatus and conditioned using EtOH and finally UHQ water. Samples were

then passed through SPE cartridges under vacuum of approximately -5 in Hg. Once samples had been passed through, cartridges were dried under the same vacuum conditions for approximately 10 min. Trapped compounds were then eluted from the cartridges with 4 mL DCM. DCM was chosen as the eluting solvent as it is known to be useful for the extraction of aroma compounds [105]. The DCM extracts were further concentrated by evaporation under a stream of nitrogen over anhydrous sodium sulphate to a volume of 0.5 mL.

2.7.3 Analytical instrumentation

One microlitre of sample was injected into a Trace GC-MS (Thermo Fisher Scientific Inc., Waltham, MA 02454, USA) equipped with a 60 m x 0.32 mm DB-Waxetr capillary column with a film thickness of 1 μm (J & W Scientific, Stockport, Cheshire, UK, SK8 3GR). The carrier gas was He at a flow-rate of 1.4 mL min^{-1}. The initial oven temperature was 60 °C, held for 1 min, increasing to 250 °C at 4 °C min^{-1} with a final hold time 10.5 min. Initial temperature of the programmed temperature vaporiser (PTV) injector was 120 °C increasing to 250 °C at 10 °C s^{-1} and held for 5 min. The split valve and septum purge were closed for 0.3 minutes. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans s^{-1}.

All peak quantifications were made on integrated single ion peaks to diminish co-elution problems.

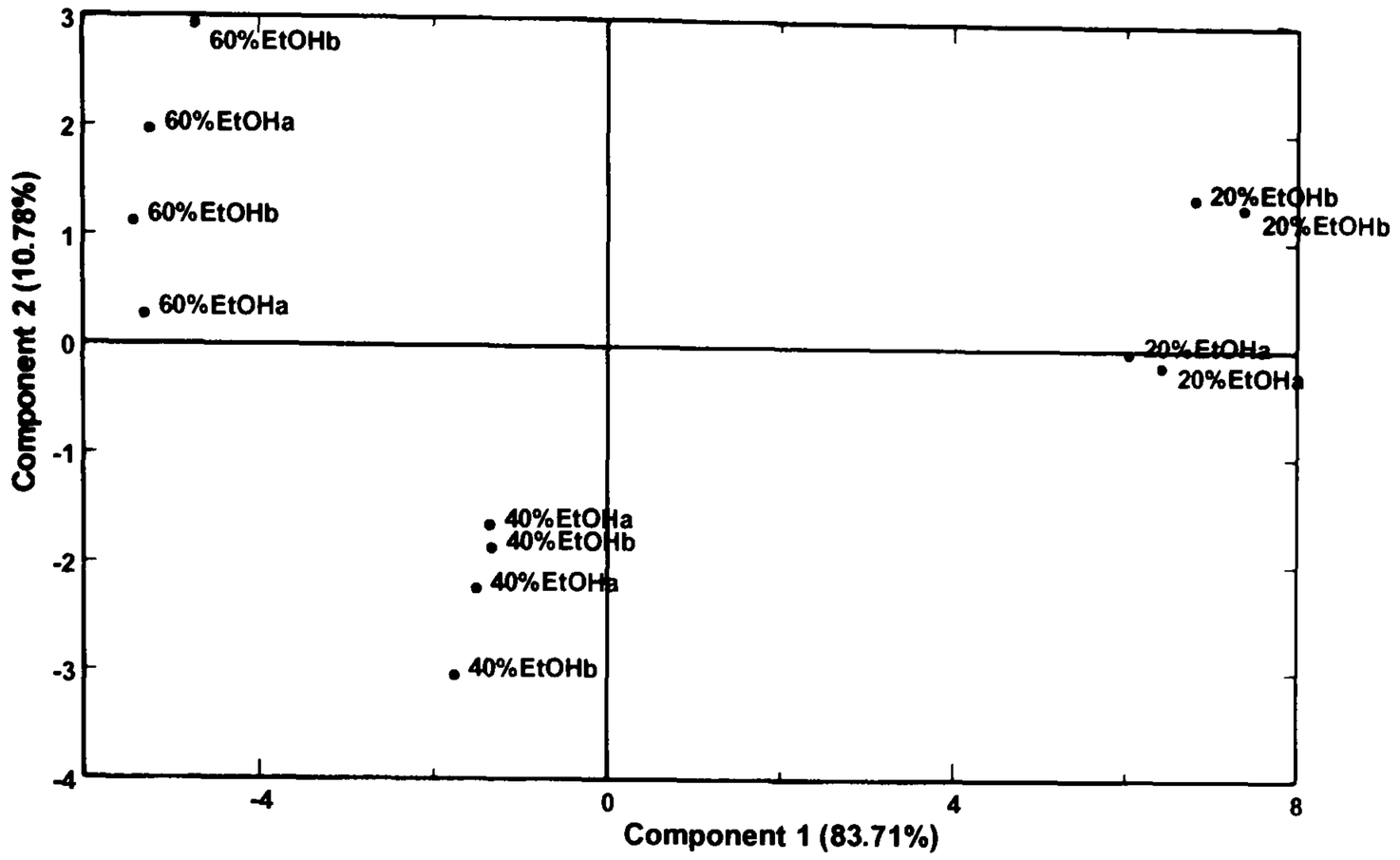
Compounds were identified as described in Chapter 2.4.2.

2.7.4 Method development

Selection of extraction solution

To determine the optimum extraction solution for peated malt, the data from the comparison of 20, 40 and 60% EtOH extraction solutions, carried out during the

development of the HS-SPME-GC-MS method for the analysis of marker phenols, were analysed.



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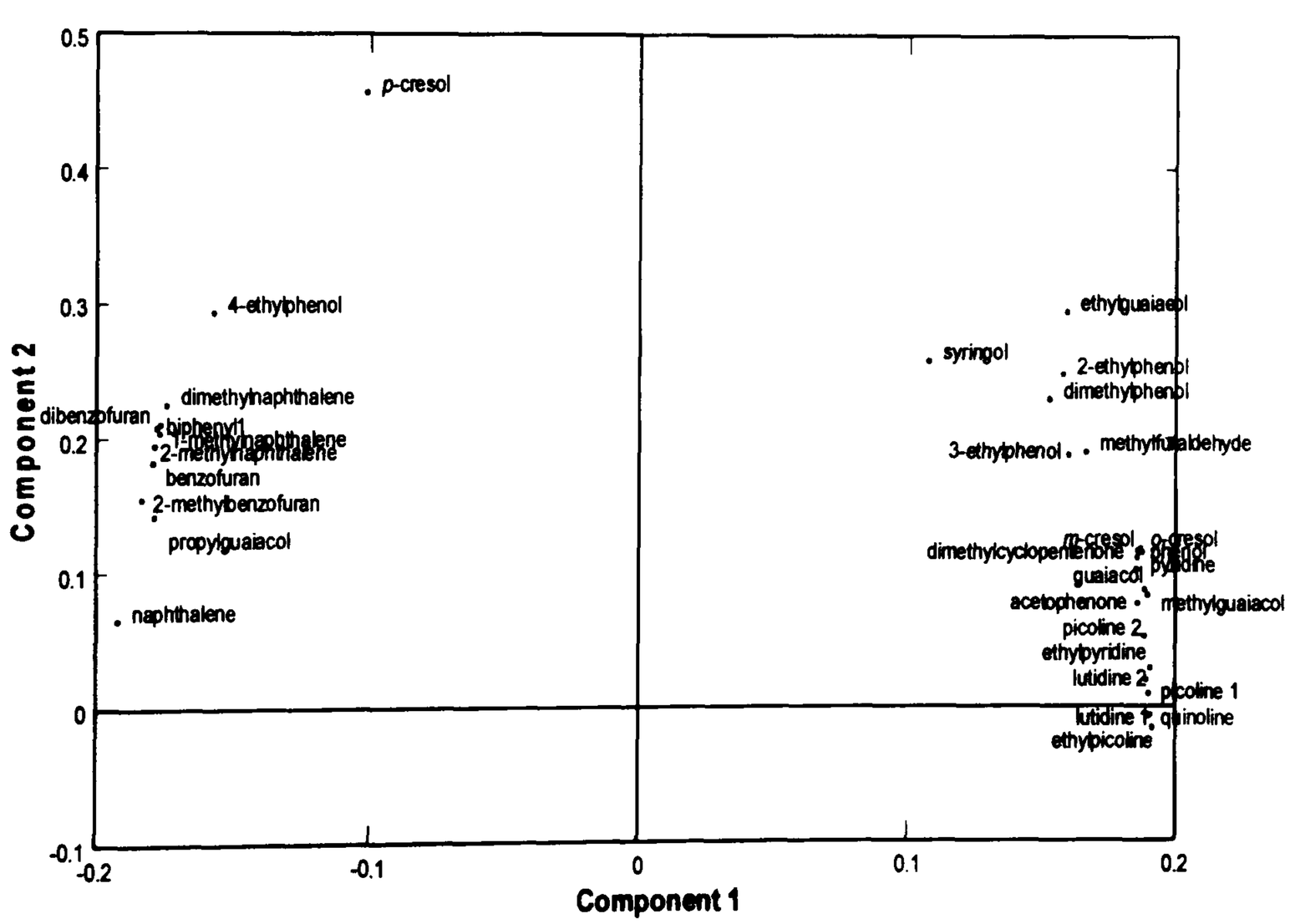


b

Figure 2.28. PCA of peak area data for various compounds analysed by HS-SPME-GC-MS using a range of ethanol concentrations. a: PCA score plot, b: loadings plot.

As shown in Fig. 2.28, it was found that the compound classes under investigation (aromatics, carbohydrate derivatives, guaiacols, nitrogen-containing compounds, phenols, and syringols) were optimally extracted at a range of ethanol concentrations. Forty percent EtOH was selected as the best overall extraction solution.

2.8 Direct Injection-GC-MS for the Analysis of Marker Phenols in Peated New-Make Spirit

2.8.1 Standards

Stock standard solutions of a range of phenols- phenol, *m*-cresol, *o*-cresol, *p*-cresol, 4-ethylphenol, guaiacol, 4-methylguaiacol and 4-ethylguaiacol - were prepared in ethanol solution. A stock standard solution of internal standard, 2, 3, 5-trimethylphenol, was also prepared in ethanol solution.

2.8.2 Sample preparation

One millilitre of new-make spirit samples, approximately 60–80% EtOH, was added to 2 mL vials. Zero point one millilitres of working internal standard solution (approximately 90 ppm 2, 3, 5-trimethylphenol in 100% EtOH) was added to all samples.

For quantitation, the eight marker phenols as identified in the HS-SPME analysis of peated malt were used. Phenol standards were made up in 60% EtOH and added to vials in 1 mL aliquots in the approximate range 0 to 20 ppm with the addition of 0.1 mL internal standard solution.

2.8.3 Analytical instrumentation

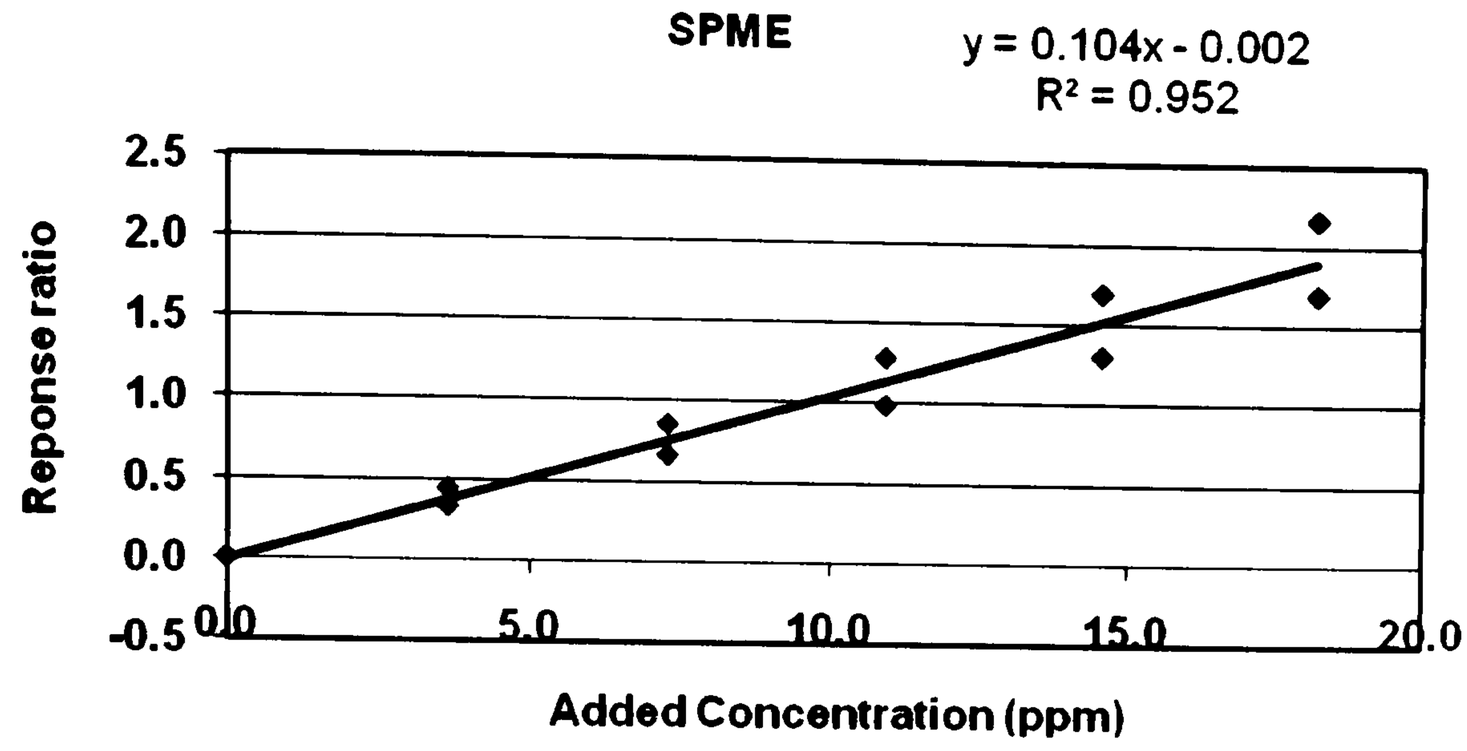
One microlitre of sample was injected into a Trace GC-MS (Thermo Fisher Scientific Inc., Waltham, MA 02454, USA) equipped with a 60 m x 0.32 mm DB-Waxetr capillary column with a film thickness of 1 μm (J & W Scientific, Stockport, Cheshire, UK, SK8 3GR). The carrier gas was He at a flow-rate of 1.2 mL min^{-1}. The initial oven temperature was 60 °C, held for 1 min, increasing to 260 °C at 4 °C min^{-1} with a final hold time 10 min. Initial temperature of the programmed temperature vaporiser (PTV) injector was 120 °C increasing to 240 °C at 10 °C s^{-1} and held for 0.5 min. The split valve and septum purge were closed for 0.5 min. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans s^{-1}.

To diminish co-elution problems, all quantifications were made on integrated quantitation ion peaks. Compound identities were obtained by analysing reference samples of the pure compounds.

2.8.4 Method development

Comparison of HS-SPME and direct injection

Initially it was hoped to use HS-SPME-GC-MS to analyse the marker phenols in new-make spirit given its advantages of sensitivity and selectivity. Therefore, this method, as detailed in Chapter 2.9, was used to determine the marker phenol levels in a range of peated and unpeated new-make spirit samples. As a comparison, these new-make spirit samples were also analysed by the existing method of direct injection into the GC-MS. The calibration curves, where responses were reported as analyte peak areas relative to internal standard peak areas, obtained at the start and the end of the analytical run were plotted for each method of analysis. The linear regression data for the two methods were compared (Fig. 2.29).



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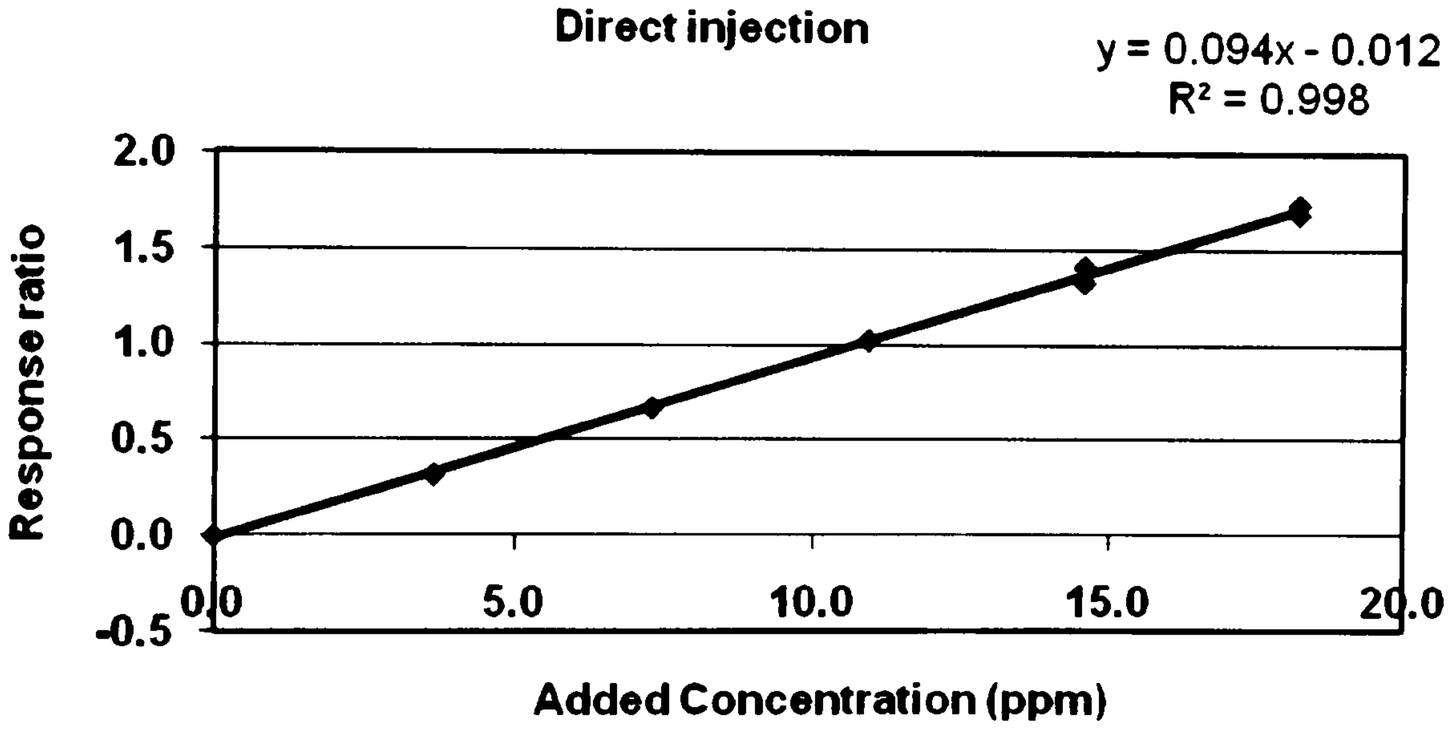


b

Figure 2.29. Calibration curves obtained for *m*-cresol using: a HS-SPME and b direct injection.

It was found that in the case of the HS-SPME method, there was a difference in the calibration curve obtained at the start and at the end of the run and this difference was not apparent in the case of the direct injection method (Fig. 2.29). The responses for the calibration curves obtained at the start and end of the run were plotted against each other

(Table 2.19). The variation in values for the slope in Table 2.19 for the HS-SPME method showed that the different groups of compounds were affected differently by time.

Table 2.19. Comparison of calibration curves at start and end of run. Curve at start of run was used as the x value and curve at end of run used as the y value.

Direct injection				**HS-SPME**			
Compound	**Slope**	**Lower 95%**	**Upper 95%**	**Compound**	**Slope**	**Lower 95%**	**Upper 95%**
Guaiacol	0.9786	0.9172	1.0400	Guaiacol	0.8567	0.8230	0.8904
Methylguaiacol	0.9904	0.9766	1.0043	Methylguaiacol	0.8915	0.8603	0.9226
o-Cresol	0.9785	0.9030	1.0540	*o*-Cresol	1.0041	0.9214	1.0868
Phenol	0.9827	0.8579	1.1075	Phenol	1.1819	1.0814	1.2825
Ethylguaiacol	0.9858	0.9578	1.0138	Ethylguaiacol	0.9513	0.8933	1.0093
p-Cresol	0.9932	0.9017	1.0847	*p*-Cresol	1.2648	1.2155	1.3141
m-Cresol	0.9972	0.9040	1.0905	*m*-Cresol	1.2792	1.2513	1.3070
4-Ethylphenol	0.9926	0.9201	1.0651	4-Ethylphenol	1.2201	1.1093	1.3310

Comparison of results

The results obtained for concentrations of the marker phenols using the two methods were compared by linear regression. For this analysis the direct injection results were assumed to be the most accurate and so the data obtained using this method was plotted as the x values whilst the HS-SPME data was plotted as the y values. The negative y intercept values for all compounds and slope values less than 1 for all compounds except 4-ethylphenol reflected the fact that HS-SPME tended to underestimate the concentrations of the marker phenols (Table 2.20).

Table 2.20. Comparison of HS-SPME and direct injection methods by linear regression results. Direct injection were x values and HS-SPME were y values.

Compound	Slope	Lower 95%	Upper 95%	Intercept	Lower 95%	Upper 95%	R^2
Guaiacol	0.6144	0.4279	0.8009	-0.2862	-0.7474	0.1749	0.75
Methylguaiacol	0.8307	0.6490	1.0124	-0.1180	-0.3578	0.1218	0.85
o-Cresol	0.8520	0.7122	0.9918	-0.2547	-0.7494	0.2401	0.91
Phenol	0.9329	0.7692	1.0967	-0.0121	-0.6800	0.6559	0.90
Ethylguaiacol	0.9736	0.8008	1.1464	-0.4468	-0.6617	-0.2318	0.90
p-Cresol	0.6520	0.5626	0.7413	-0.0897	-0.3747	0.1952	0.94
m-Cresol	0.9410	0.8172	1.0648	-0.1342	-0.2863	0.0179	0.94
4-Ethylphenol	1.1928	1.1532	1.2324	-0.7102	-0.7776	-0.6428	1.00

It was also noted that some of the R^2 values were relatively low, especially in the case of guaiacol (Table 2.18). The reason for this discrepancy was investigated.

Effect of esters on HS-SPME response

It was postulated that esters, such as ethyl decanoate and ethyl dodecanoate, which are highly abundant in whisky may affect the uptake of the marker phenols onto the SPME fibre. Therefore, the new-make spirit samples were analysed for their ester content (Table 2.21). There was found to be a large range of concentrations of the various esters analysed. Notably, the two Swedish samples were found to contain particularly low concentrations of the ethyl esters analysed.

Table 2.21. Results of esters analysis (values are in ppm).

Sample	Ethyl Hexanoate	Ethyl Octanoate	Ethyl Decanoate	Ethyl Dodecanoate	Ethyl Tetradecanoate	Ethyl Hexadecanoate
Caithness	1.6	7.4	26.4	21.3	4.7	13.1
Invernesshire	1.1	5.1	14.4	12.3	2.5	10.6
Islay a	0.5	2.9	6.7	4.3	1.2	9.5
Islay b	1.0	5.3	16.3	11.8	2.3	8.7
Islay c	0.9	5.2	16.6	14.4	4.2	15.9
Islay d	1.8	9.7	31.3	26.5	5.4	12.8
Islay e	1.4	6.6	23.9	22.5	4.9	15.0
Islay f	0.9	5.0	16.2	8.5	1.0	7.0
Orkney	1.6	7.2	24.9	19.9	3.6	10.6
St Fergus a	1.7	10.5	33.5	29.8	5.9	16.6
St Fergus b	2.4	13.3	45.0	38.9	15.2	15.5
St Fergus c	2.1	12.8	44.6	36.5	9.6	13.0
St Fergus d	1.8	10.0	34.6	31.9	7.6	16.6
St Fergus e	1.2	6.3	19.7	16.5	3.8	10.5
St Fergus f	0.5	2.7	8.9	6.6	2.0	15.8
Sweden a	0.7	2.6	5.0	2.5	0.0	2.6
Sweden b	0.6	2.4	4.3	2.1	0.0	1.9
Unpeat	1.3	7.4	24.6	22.8	4.6	15.8

The residual values from the regression line fitted to the plot of HS-SPME results against direct injection results for guaiacol concentration (regression line data in Table 2.20) were related to the ester concentrations by using PCA (Fig. 2.30). This was done to determine if the ester concentrations had an effect on the concentration of this compound as determined using HS-SPME. Also included in this analysis was the peak area data for the internal standard (2, 3, 5-trimethylphenol) for each sample analysed using the HS-SPME method.

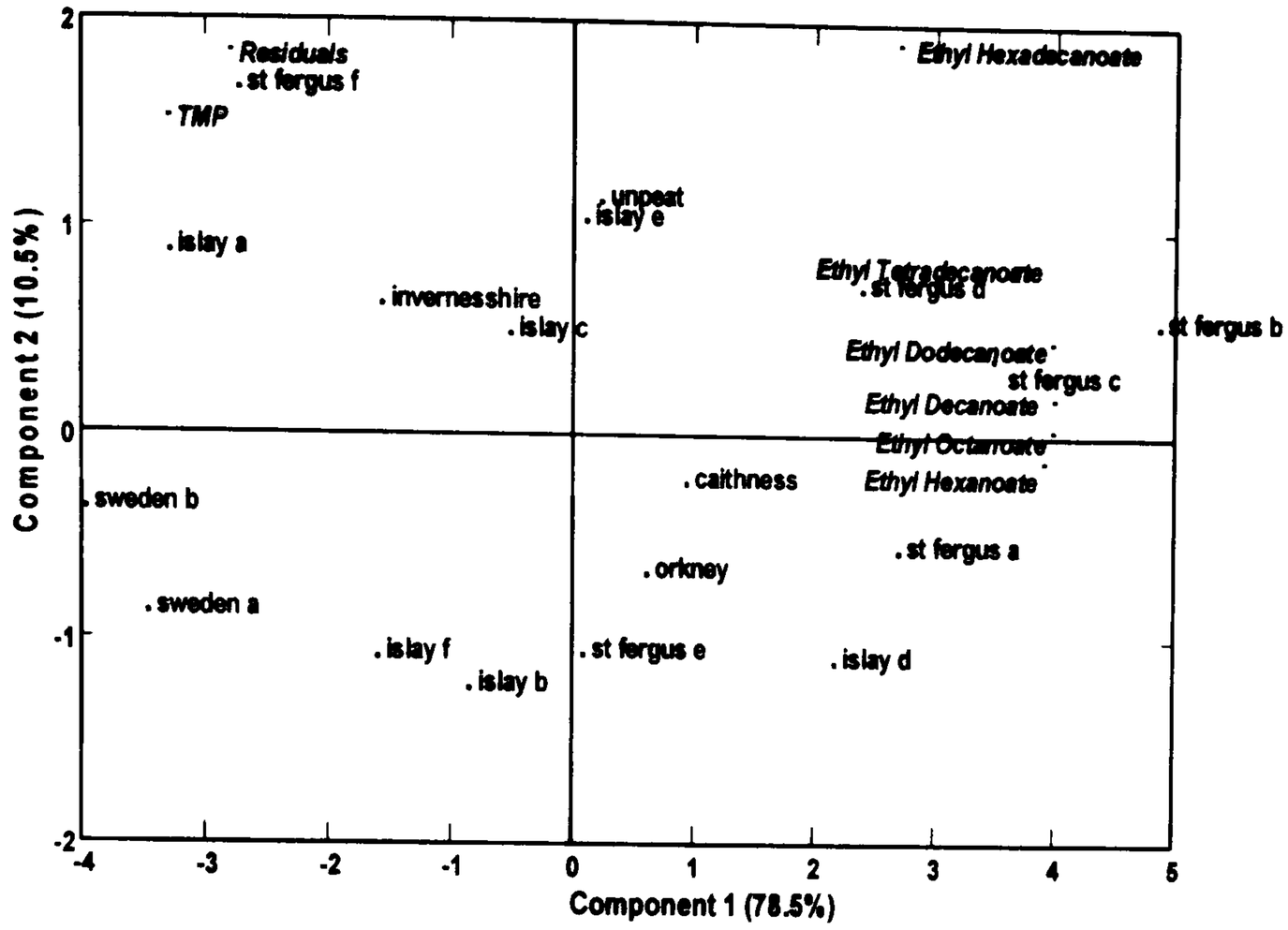


Figure 2.30. PCA bi-plot comparing new-make spirits in terms of HS-SPME v direct injection regression residuals for guaiacol (labeled Residuals), esters concentrations and 2, 3, 5-trimethylphenol peak area.

Figure 2.30 shows that 2, 3, 5-trimethylphenol peak area correlated well with the regression residual values. PC 1 shows that samples with a high level of esters generally gave low residual values and low 2, 3, 5-trimethylphenol peak areas. This suggested that esters were preventing marker phenols from being adsorbed by the SPME fibre. PC 2 shows that a particularly high level of ethyl hexadecanoate in the new-make spirit could limit the negative effect that the esters were having on the uptake of marker phenols by the SPME fibre.

The effect esters were having on marker phenols uptake was demonstrated in a subsequent experiment where increasing concentrations of three ethyl esters (ethyl decanoate, ethyl dodecanoate and ethyl hexadecanoate) were added to a standard solution of marker phenols (approximately 4 ppm of each phenol). The results of this experiment showed that increasing levels of ethyl decanoate and, even more so, ethyl dodecanoate had a negative influence on marker phenols peak areas (Fig. 2.31 and Table 2.22). Ethyl hexadecanoate did not appear to influence the recovery of the marker phenols.

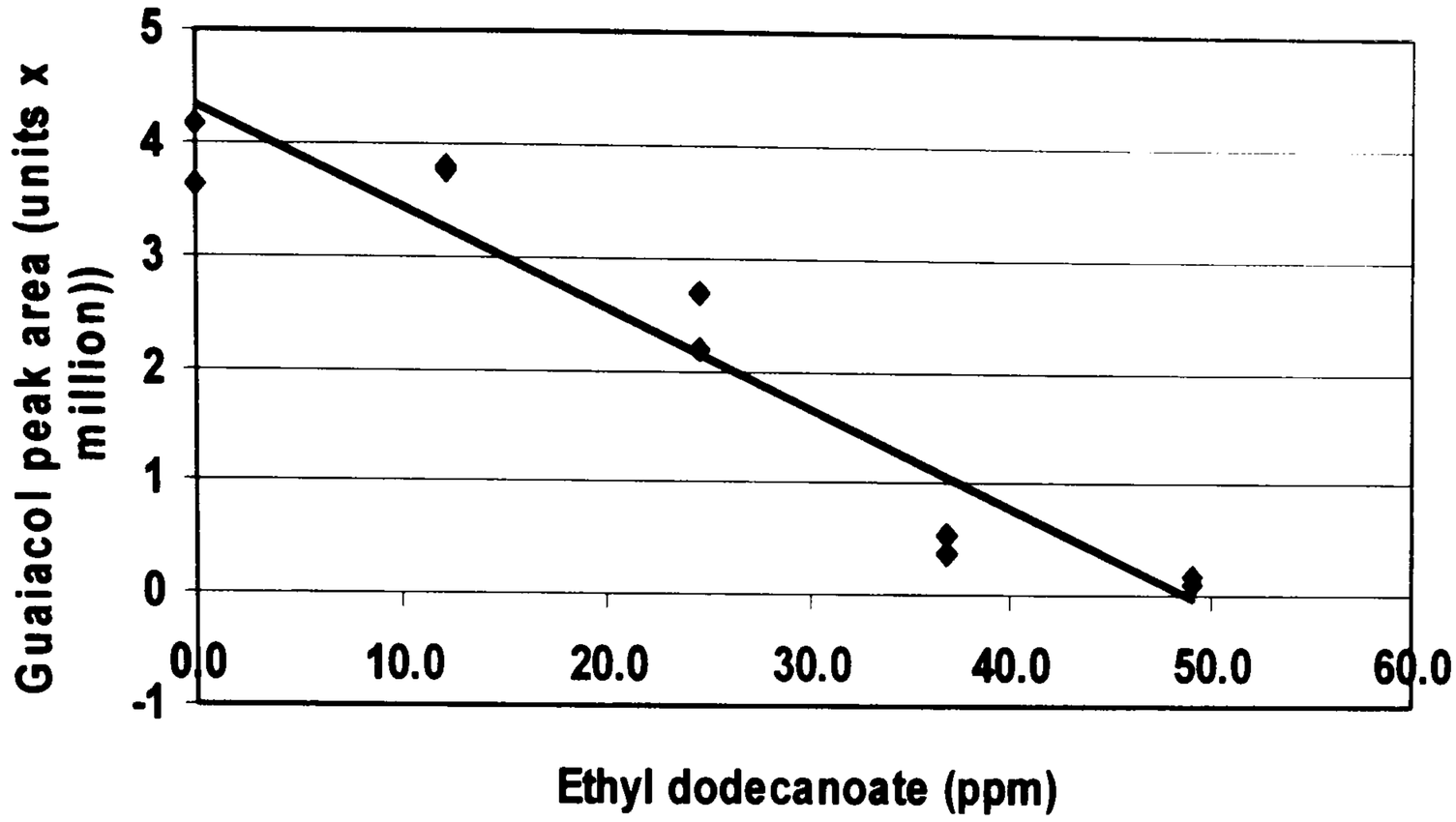


Figure 2.31. Guaiacol peak area at different ethyl dodecanoate concentrations.

Table 2.22. Correlation coefficients of marker phenol peak areas against increasing ester concentrations.

Compound	Guaiacol	Methylguaiacol	*o*-Cresol	Phenol	Ethylguaiacol	*p*-Cresol	*m*-Cresol	4-Ethyl phenol
Ethyl decanoate	-0.92	-0.87	-0.84	-0.74	-0.84	-0.77	-0.75	-0.73
Ethyl dodecanoate	-0.96	-0.95	-0.96	-0.95	-0.95	-0.95	-0.95	-0.95
Ethyl hexadecanoate	0.00	0.00	0.00	0.01	-0.08	0.00	0.02	-0.01

It appeared therefore, to be the relatively volatile esters that were having the most effect on the marker phenols responses and so it was suspected that the interference was taking place in the headspace rather than in solution.

To confirm that the esters were having an effect in the headspace, an experiment was designed where a series of marker phenol standards was analysed using the standard HS-SPME method and then this analysis was repeated after the SPME fibre was exposed to the headspace of a 20 ppm solution of ethyl decanoate and ethyl dodecanoate. This experiment showed that coating the SPME fibre with ethyl esters had an adverse effect on the response of phenols (Table 2.23 and Fig. 2.32).

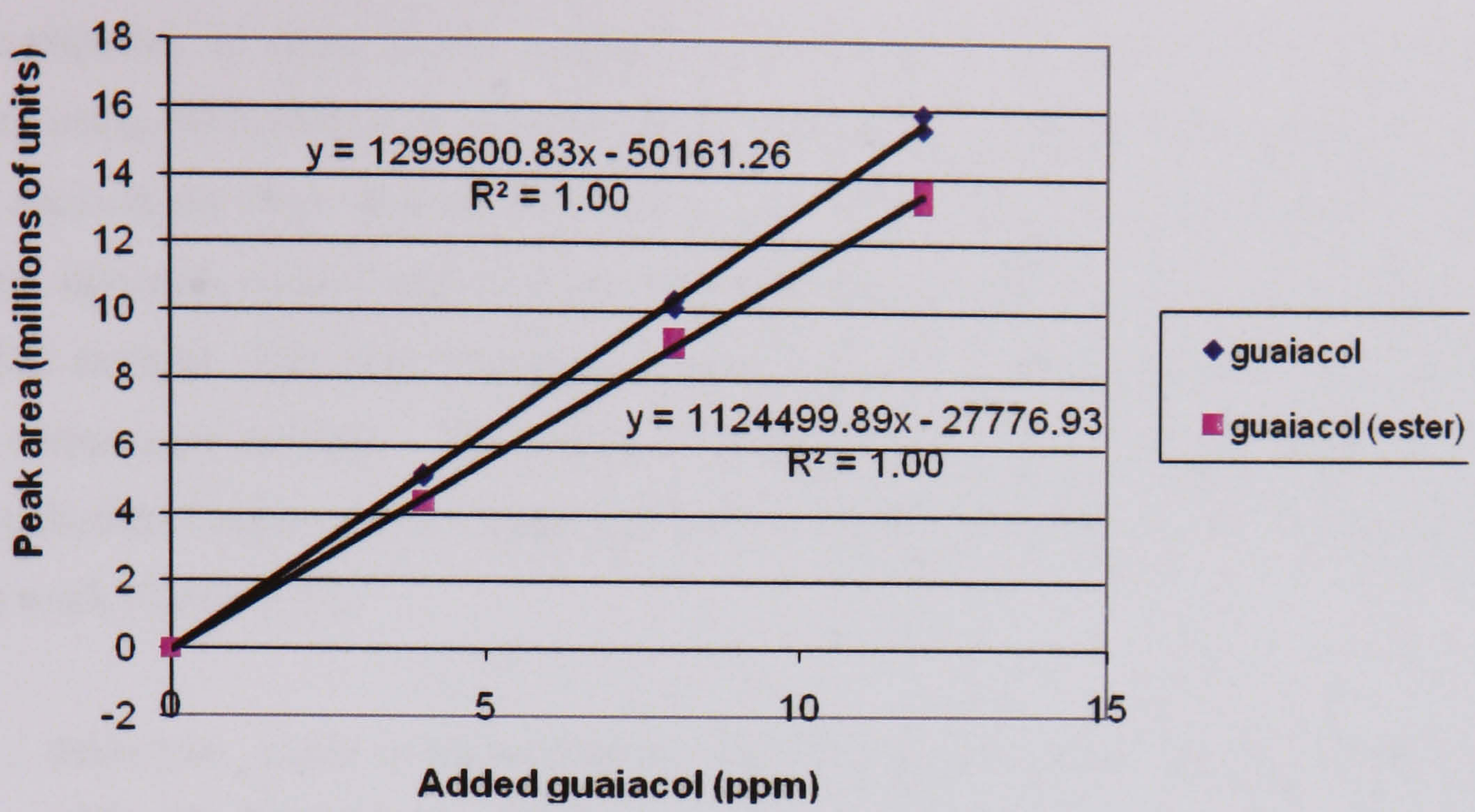


Figure 2.32. Guaiacol peak area in the presence and absence of a 20 ppm solution of ethyl decanoate and ethyl dodecanoate.

Table 2.23. Effect of pre coating SPME fibre with esters on marker phenols response.

Sample ID	**Slope in presence of esters/ slope with no esters (%)**
Guaiacol	87
Methylguaiacol	82
o-Cresol	88
Phenol	80
Ethylguaiacol	87
p-Cresol	79
m-Cresol	79
4-Ethylphenol	83

Method selection

The presence of esters in the headspace reduced the recoveries of the marker phenols when using HS-SPME and, most notably in the case of guaiacol, the internal standard did not account for these fluctuations. As a result of this finding it was decided to use the direct injection method and to avoid the problems found to be associated with the HS-SPME method. The direct injection method calibration curves showed good linearity for all compounds studied. The limits of detection were determined using the method described in Chapter 2.5.4. These values were found to be adequate for the purposes of this work (Table 2.24).

Table 2.24. Limits of detection for the analysis of marker phenols in peated new-make spirit using direct injection-GC-MS.

Compound	**LOD (ppm)**
Guaiacol	0.38
Methylguaiacol	0.34
o-Cresol	0.20
Phenol	0.19
Ethylguaiacol	0.26
p-Cresol	0.21
m-Cresol	0.15
4-Ethylphenol	0.22

2.9 HS-SPME-GC-MS for the Analysis of Marker Phenols in Peated New-Make Spirit

2.9.1 Standards

The same standards were used as for Chapter 2.8.1.

2.9.2 Sample preparation

Two millilitres of new-make spirit samples, approximately 60% EtOH, were added to 10-ml headspace vials and diluted to approximately 20% EtOH with 4 mL UHQ water. Zero point two millilitres of working internal standard solution (approximately 90 ppm 2, 3, 5-trimethylphenol was added to all samples.

For quantitation, the eight marker phenols as identified in the HS-SPME analysis of peated malt were used. Phenol standards were made up in 60% EtOH and added to headspace vials in 2 ml aliquots in the approximate range 0 to 20 mg L^{-1}. Standard solutions were then diluted to 20% EtOH with UHQ water.

Headspace sampling conditions were optimised previously for the recovery of the marker phenols. The fibre used was: 85 µm polyacrylate (PA) (Supelco UK, Gillingham, Dorset, UK, SP8 4XT). Sample pre-incubation time was 20 min at 50 °C. Extraction time was 15 min.

2.9.3 Analytical instrumentation

The analytical method used was the same as that described in Chapter 2.6.3 for the analysis of peated malt extracts.

2.10 Determination of Esters Levels in Spirit by GC-FID (Gas Chromatography-Flame Ionisation Detection)

2.10.1 Standards

Stock standard solutions of a range of esters- ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexanoate and ethyl tetradecanoate - were prepared in 70% EtOH/ H_2O. Ethyl hexadecanoate was prepared in 100% EtOH. The internal standard, methyl octadecanoate, was also prepared in 100% EtOH.

2.10.2 Sample preparation

Calibration standards were prepared in the range: 1 to 50 ppm. One millilitre of standard was added to a 2-mL auto sampler vial along with 0.1 mL of working internal standard solution (100 ppm in EtOH).

Spirit samples were adjusted to 70% v/v alcohol strength by adding 0.5 mL of sample to 2-mL auto sampler vials along with 0.5 mL of an appropriate EtOH solution. Zero point one millilitres of working internal standard solution was added to this.

2.10.3 Analytical instrumentation

Analyses were performed with a Hewlett-Packard 6890 gas chromatograph fitted with an flame ionisation detector (FID) (Agilent Technologies UK Limited, Stockport, Cheshire, UK, SK8 3GR). The column used was a 60 m x 0.32 mm DB-Waxetr capillary column with a film thickness of 1 μm (J & W Scientific, Stockport, Cheshire, SK8 3GR, UK). Half a microlitre of sample was injected in splitless mode. The carrier gas was H_2 and pressure was set to 17.10 psi (giving a flow-rate of 3.4 ml min^{-1} at 100 °C). The initial oven temperature was 35 °C, held for 3 min, increasing to 130 °C at 20 °C min^{-1} then increasing to 190 °C at 10 °C min^{-1} and holding for 5 min then increasing to 240 °C at

10°C min^{-1} with a final hold time of 33.25 min. The injector temperature was maintained at 240 °C. Detector temperature was maintained at 250 °C.

2.11 Analysis of New-Make Spirit by GC-Olfactometry/MS (GC-O/MS)

2.11.1 Standards

Stock standard solutions of the internal standards - 2, 3, 5-trimethylphenol, naphthalene-d8 and pyridine-d5 were prepared in EtOH.

2.11.2 Sample preparation

Ten millilitres of new-make spirit sample was measured out and diluted to 20% EtOH with UHQ water. The internal standards were added, relative to the new-make spirit, at the following concentrations: 2, 3, 5-trimethylphenol, 4 ppm; naphthalene- d8, 0.2 ppm and pyridine- d5, 1 ppm.

Phenomenex Strata-X SPE cartridges (Phenomenex, Macclesfield, Cheshire, UK, SK10 2BN), with a sorbent mass of 200 mg and reservoir volume of 3 mL, were conditioned prior to use. Cartridges were conditioned firstly with DCM then EtOH and finally equilibrated using UHQ water. Samples were then passed through SPE cartridges under vacuum at a rate of approximately 2 mL min^{-1}. Once samples had been passed through, cartridges were dried under the same vacuum conditions for approximately 10 min. Retained compounds were then collected with 4 mL of EtOH. One millilitre of extract was added to a 2-mL auto sampler vial prior to analysis by GC-O/MS or GC-MS.

2.11.3 Analytical instrumentation

GC-O/MS

The extracts were analysed by a 2 µl splitless injection on a Hewlett-Packard 5890 series II gas chromatograph (Agilent Technologies UK Limited, Stockport, Cheshire, UK, SK8 3GR). The flow from the gas chromatograph was split in the approximate ratio 6:1 between a Gerstel ODP2 olfactory detection port fitted with a glass sniffing cone (GERSTEL GmbH & Co.KG, D-45473 Mülheim an der Ruhr, Germany) and a 5971 mass spectrometer (Agilent Technologies UK Limited, Stockport, Cheshire, UK, SK8 3GR). The sniffing cone was purged with humidified air to help in maintaining olfactory sensitivity by reducing dehydration of mucous membranes in the nasal cavity. The column used was a 60 m x 0.32 mm DB-Waxetr capillary column with a film thickness of 1 µm (J & W Scientific, Stockport, Cheshire, UK, SK8 3GR). The carrier gas was He and the head pressure was 23 psi (giving a flow-rate of 2.3 mL min^{-1} at 100 °C). The initial oven temperature was 40 °C, held for 1 min, increasing to 250 °C at 5 °C min^{-1} with a final hold time of 10 min. The injector temperature was maintained at 240 °C throughout the analysis. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans per second.

Two assessors participated in perceiving the aroma compounds derived from peated and unpeated new-make spirit samples at the sniffing port. The two assessors were members of the SWRI's internal sensory panel who had undergone extensive sensory training and had substantial experience in the assessment of new-make spirits. Each assessor carried out the analysis of each sample in duplicate, samples were analysed blind and in a random order. The sniffing time for each run was 40 min. Assessors were asked to record the time at which they could first detect an odour and give a qualitative description of that odour.

Compounds were identified as described in Chapter 2.4.2.

To determine which compounds were organoleptically most important, aroma extraction dilution analysis (AEDA) was carried out by one of the two assessors. AEDA was performed by injecting, at incremental dilutions, a single new-make spirit extract. The dilutions values used were 10, 100 and 1000. Each dilution was analysed in duplicate and samples were analysed blind and in a random order. The highest dilution value at which odours were detected was recorded. Odours which were detected in the diluted extracts but not in the undiluted extract were discounted as noise.

GC-MS

The analytical method used was the same as that described in Chapter 2.8.3 for the analysis of whole new-make spirits.

2.11.4 Method development

Selection of SPE cartridge

A lab-scale peated new-make spirit (produced using Castlehill peat) was extracted using a Strata-X SPE cartridge (Phenomenex, Macclesfield, Cheshire, UK, SK10 2BN) with a sorbent mass of 200 mg and reservoir volume of 3 mL and a Supelco Discovery C18 SPE cartridge with a sorbent mass of 1000 mg and reservoir volume of 6 mL (Supelco, Gillingham, Dorset, UK, SP8 4XT). Both cartridges were conditioned in the same way prior to use. Cartridges were conditioned firstly with EtOH and finally equilibrated using UHQ water. In each case, 8 mL of sample was diluted to approximately 20% EtOH by dilution with 22 mL of UHQ water. Samples were then passed through SPE cartridges under vacuum at a rate of approximately 2 mL min^{-1}. Once samples had been passed through, cartridges were dried under the same vacuum conditions for approximately 10 min. Retained compounds were then collected with 6 mL of EtOH which was subsequently diluted to 20% EtOH with 24 mL of UHQ water.

A comparison of the aromas of the extracts obtained using the Strata-X and C18 SPE cartridges were made with the aroma of the original spirit by five members of the SWRI sensory panel. This comparison showed that the extract obtained using the Strata-X

cartridges gave the best representation of the original peated whisky. It was also noted that in the unretained fraction obtained from the C18 extraction, there was a residual sweet/ caramel aroma.

Subsequently, the Strata-X and C18 extracts were compared to the original spirit sample using HS-SPME-GC-MS (Fig. 2.33).

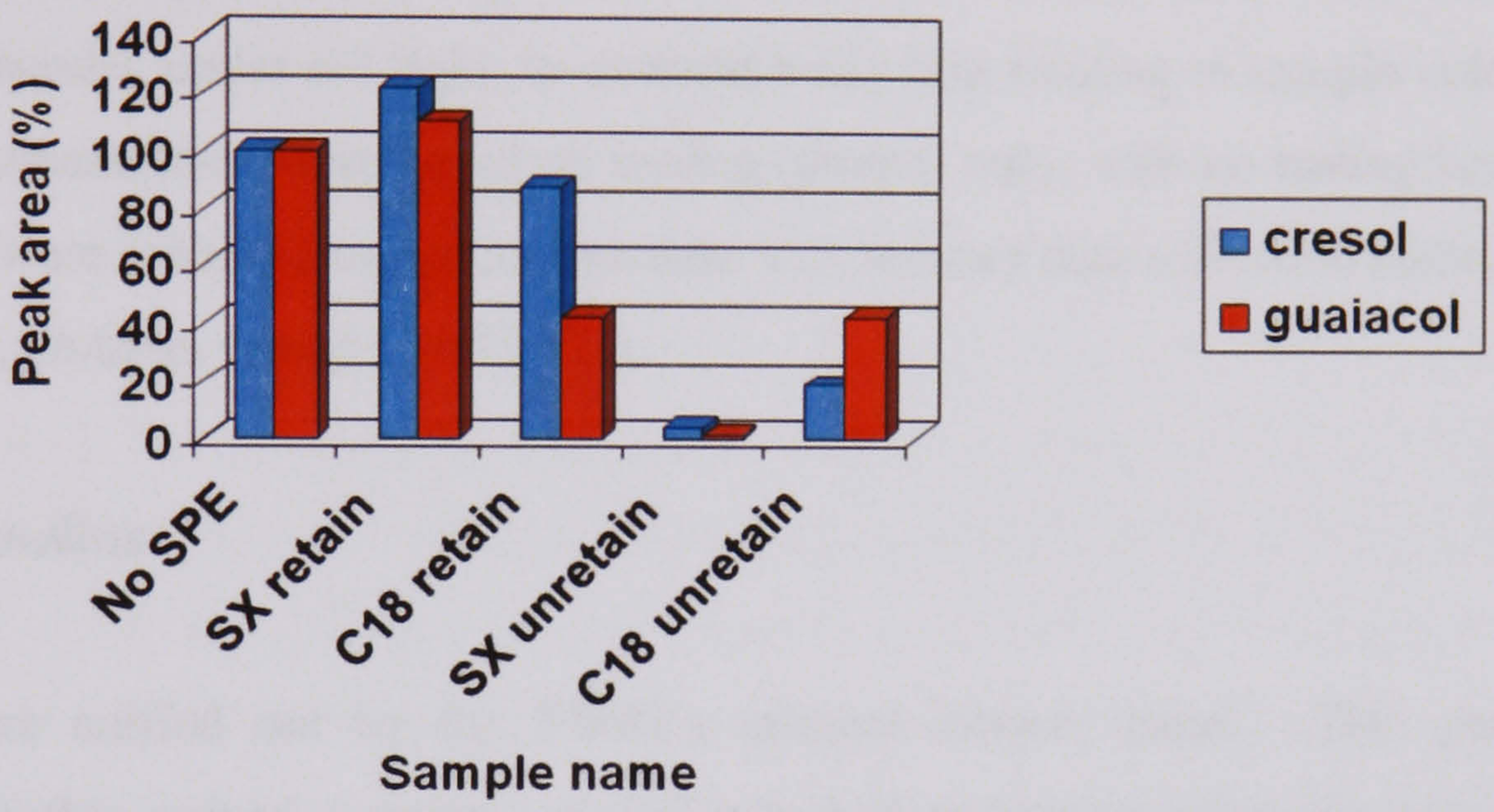


Figure 2.33. Peak areas for compounds either retained by Strata-X (SX) or C18 SPE columns or unretained. Also shown are data for the untreated spirit sample (No SPE). Data are relative to the peak area in the untreated spirit sample.

It was found that for these two peat-derived compounds, Strata-X performed best, particularly in the case of guaiacol. This may provide a possible explanation for the residual sweet/ caramel aroma in the unretained fraction from the C18 extraction as guaiacol and its relatives are known to have an aroma matching this description [106].

2.12 Sensory Analysis

2.12.1 Sample preparation and presentation

The lab-scale spirits were reduced to 20% abv using well run tap water. Twenty millilitre portions presented in clear 130-mL nosing glasses were used and were covered with 50-mm watch glasses in order to retain a headspace. Samples were identified using three figure random codes and presentation order was randomised. Assessments were carried out in individual booths, under red light, to overcome any bias relating to sample colour or turbidity. All assessments were based on nosing (aroma) only, with no tasting being carried out. Data were collected using Compusense V.5, sensory data collection software (Compusense Inc., Ontario, Canada, N1G 4S2).

2.12.2 Sensory panelists

Sensory tests were carried out by the SWRI's internal sensory panel. This panel comprised of 22 highly trained members of staff who had undergone extensive sensory training and had substantial experience in the assessment of new-make spirits. A minimum of 10 panelists participated in each sensory session.

2.12.3 Exploring differences in aroma using Triangle Tests

There are a number of standard sensory methods that can be used to determine whether or not differences exist between samples. The method chosen for use in this study was the Triangle Test, carried out in accordance with British Standard BS 4120: 2004 [107]. In each test three samples were presented, two of one kind and a third "odd" sample. The assessor was asked to identify this "odd" sample. The Triangle Test was carried out as a forced-choice test, with the assessor being asked to guess if they do not know the correct answer.

Data were analysed by determining whether or not the number of correct responses was significantly greater than the number likely to be obtained through chance. This analysis was carried out using the Compusense software. A probability value (p value) of <0.05 was interpreted as a significant sensory difference. This equates to a percentage confidence of >95% that a difference exists between samples.

2.12.4 Measurement of aroma differences using Quantitative Descriptive Analysis

Quantitative Descriptive Analysis, another well establish sensory method, was used to provide a measure of the relative intensity of a range of pre-determined sensory attributes. This test was set-up in accordance with British Standard BS 13299:2003 [108]. The attributes studied were the peaty aromas listed on the whisky flavour wheel (See Fig. 1.2), namely: intensity of peaty aroma, burnt, medicinal and smoky. Also, the presence of "other new-make spirit aromas" was used as a single attribute to define any additional aromas. Each test sample was presented to the assessor, who was asked to score them in terms of intensity of each attribute. Scores were given on a line scale of 0–3 with intervals of 0.1. When carrying out Quantitative Descriptive Analysis assessors were forced to give a score for each attribute, even if this was zero, to ensure that attributes were not inadvertently omitted. Data were collated and exported to Excel and ANOVA was used to explore whether or not there were significant differences between samples for each attribute. Average scores across the panel were calculated and Principal Components Analysis was used to examine the relative distribution of samples.

2.13 Lab-Scale Peated Malt Production

2.13.1 Materials

Green malt samples were supplied by North British distillery and were stored in a deep freeze at -30 °C. The yeast used was pressed 'M' Type and was supplied by Kerry Bio-Science (Menstrie, Clackmannanshire, UK, FK11 7ES).

The peat used was a composite of representative 20 g ± 0.1 g sub-samples obtained from each of the transect samples collected from each of the six sampled peat sources. In this way, the source of the samples was traceable. These sub-samples were obtained using a sample divider (Endecotts Limited, London, UK, SW19 3TZ). Sub-samples were then combined and homogenised.

2.13.2 Moisture contents

Green malt moisture content

This method is a modification of Institute of Brewing (IOB) method 1.2 [109]. Approximately 20 g green malt was weighed in a metal weighing dish and oven dried at 130 °C for 1.5 h. The sample was then placed in a dessicator to cool and reweighed. The moisture content was calculated by subtracting the dry malt weight from the wet weight and was reported as a percentage of the original wet weight (moisture % **1**).

The dried malt was milled using a Buhler Miag Universal Disc Mill (Buhler GmbH, Braunschweig, Germany, Post fach 3369, D-38023) which was set with a gap width of 0.2 mm using a feeler guage. Approximately 10 g of this ground malt was weighed in a metal weighing dish and oven dried at 130 °C for 1 h. The sample was again placed in a dessicator to cool and was subsequently reweighed. The moisture content was calculated as before (moisture % **2**). The total moisture content of the malt sample was calculated using the following equation:

$$\text{Green malt moisture content (\%)} = (\mathbf{1} + \mathbf{2}) - ((\mathbf{1} \times \mathbf{2})/100) \qquad (2)$$

Kilned malt moisture content

This method is a modification of IOB method 1.2 [109]. Approximately 10 g of malt was milled using a Buhler Miag Universal Disc Mill (Buhler GmbH, Braunschweig, Germany, Post fach 3369, D-38023) (gap width of 0.2 mm using a feeler guage). Approximately 5 g of ground malt was weighed in a metal weighing dish and oven dried

at 105 °C for 3 h. The sample was placed in a dessicator to cool and reweighed. The moisture content was calculated by subtracting the dry malt weight from the wet weight and was reported as a percentage of the original wet weight.

2.13.3 Predicted spirit yield (PSY) of malt

This method is a modification of IOB method 2.16 [110].

Milling

Fifty one grams of dried malt was milled using a Buhler Miag Universal Disc Mill (gap width of 0.7 mm using a feeler guage) (Buhler GmbH, Braunschweig, Germany, Post fach 3369, D-38023) and the weight of the resulting grist was adjusted 50 g ± 0.0005 g.

Mashing

Mashing was carried out in a Lochner LB 8 Electronic mashing bath (Lochner Labor and Technik GmbH, Bayreuth, Germany, D-95445) at 65 °C. The grist was transferred to a steel mashing beaker as quantitatively as possible and placed in the mashing bath to preheat for 10–15 min. Two hundred and sixty millilitres ± 10 mL distilled water at 68 °C was added to the grist and thoroughly mixed with a glass rod to break up the lumps. The glass rods and the grist containers were rinsed with a further 100 mL of distilled water at 68 °C. The mashing bath stirrers were switched on and the samples were allowed to mash for 1 h at 65 °C. Subsequently, the mashing bath water was cooled to approximately 20 °C and 25 min after starting the cooling cycle the sample beakers were removed from the water bath. The mash was made up to 450 g in a tared flask. After shaking thoroughly for 3–5 min the mash was transferred to a 32-cm diameter fluted filter paper (Whatman 54 (VWR International Ltd, Lutterworth, Leicestershire, UK, LE17 4XN) or Ederol 12 (H Rudebeck & Company, Burgess Hill, West Sussex, UK, RH15 9LH) and the filtrate collected. The first 50–70 mL of wort was returned to the filter. A small aliquot of the wort was taken in order to measure the density using the Anton Paar DMA 55 density meter (Anton Paar Scientific Ltd., Hertford, Herts, UK, SG13 7NW) enabling the determination of original gravity (OG) and the hot water extract.

Fermentation

Approximately 150 mL of the filtered wort samples were collected and added to fermentation flasks along with yeast in proportion to give a pitching rate of 0.5 g pressed 'M' type yeast per 100 mL wort. Flasks were placed in a water bath at 33 °C (± 0.2 °C) and the wort allowed to ferment for 44 h. Subsequently, the wash was cooled to ambient temperature and filtered through a fluted Whatman 2V (32 cm) filter paper (VWR International Ltd, Lutterworth, Leicestershire, UK, LE17 4XN) returning the first 50 mL to the filter. When about 100–150 mL of clear filtrate was collected, the density of the filtered wash was measured using the density meter. The density of the wash was used to determine the specific (final) gravity (FG) and subsequently the potential spirit yield.

Calculations

original gravity (OG) or final gravity (FG) = (Density (gcm^{-3})/ 0.99715) x 1000 (3)

Hot water extract (HWE) (%) = ((OG - 1000) x 2.278)/ (OG/1000) (4)

Fermentability (%) = ((OG - FG)/ OG) x 81.4 (5)

Fermentable extract (%) = (HWE (%) x Fermentability (%))/ 100 (6)

Predicted spirit yield (PSY) (litres of alcohol per tonne) = Fermentable extract x 6.06 (7)

2.13.4 Peating

The following is the final method used for peating malt on a laboratory scale. The method development is described in Chapter 2.13.6. To simulate the set up found in an industrial kiln for the production of a typical peated malt, 50 g of dried peat was burned per 750 g of green malt. Also, 25 mL of UHQ water was mixed with this peat so that combustion would occur in the presence of moisture levels similar to those found in the

industrial setting. The apparatus used for peating is shown in Fig. 2.34. Peating was carried out for 30 min.

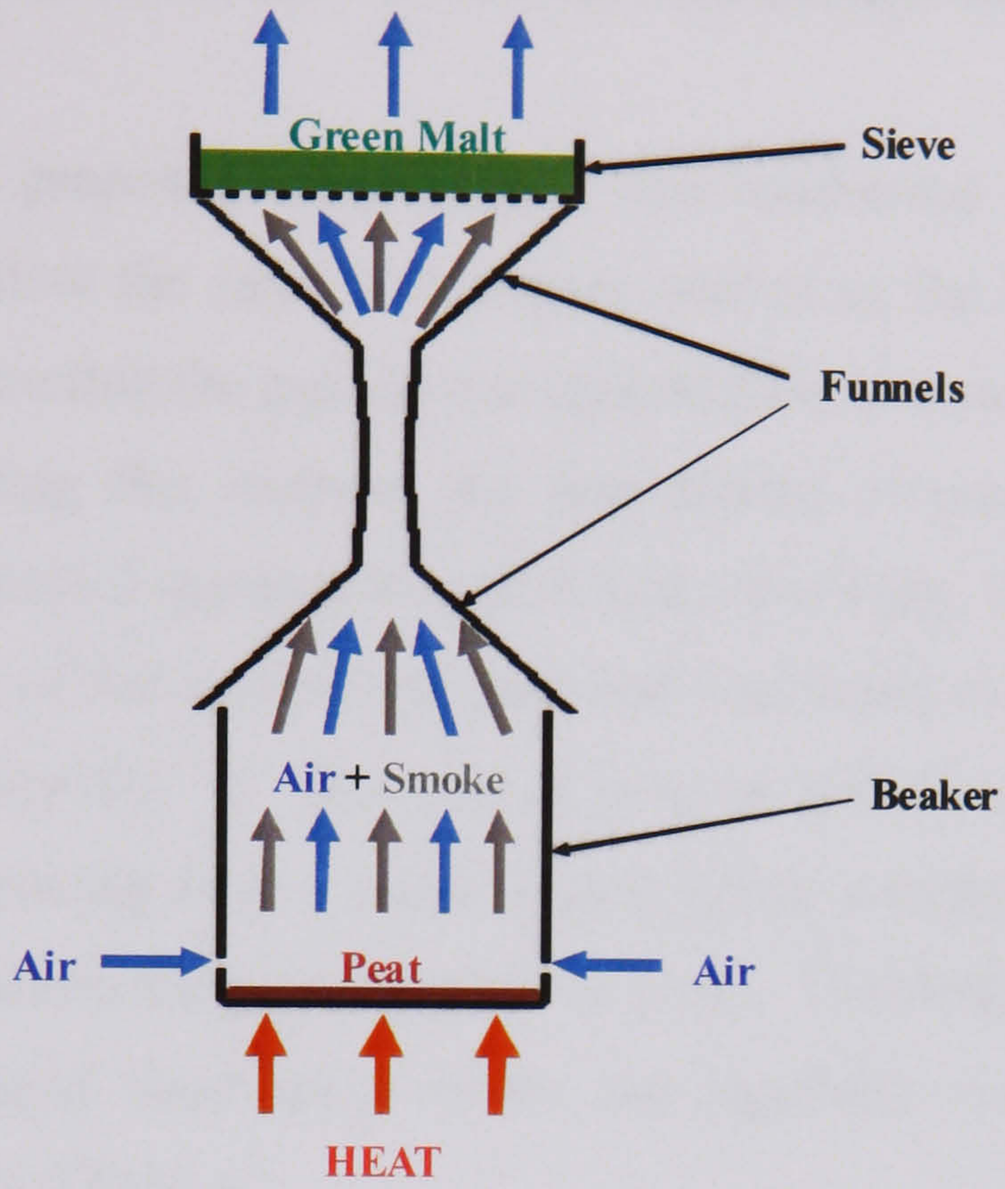


Figure 2.34. Apparatus used for the lab-scale peating of malt. Air flow was provided by placing the apparatus in a fume hood with an air flow of 0.88 m s^{-1}.

2.13.5 Kilning

Once peated, samples were kilned for 24 h in a Seeger micro kiln (Schmidt-Seeger AG, 92339 Beilngries, Germany) at a temperature of 50 °C with an airflow of 1.2 m s^{-1}. The resultant peated malt had a moisture content of approximately 6.8%.

2.13.6 Peating method development

Peating method selection

Kilning facilities were available to dry malted barley. The micro kiln did not, however, have a facility for peating so an alternative for this process was sought. It was initially hoped to use a closed furnace to burn the peat and collect the resultant smoke on the malt

as it passed out the furnace exhaust. However, when a few grams of peat, dry or with some UHQ water added, were put in the furnace and subjected to a temperature of 600 °C to replicate the conditions used for the pyrolysis of peat, no smoke was produced from the vent. This was found to be due to the very low air flow through the furnace.

An alternative set up was proposed whereby peat was combusted over a natural gas burner. This would not allow the same temperature control as the furnace. The only level of control was to ensure that the gas tap was opened fully to ensure that a consistent flame was produced. Using this method, the temperature of peat combustion was measured using a Microtherma 2 thermometer (ETI Ltd., Worthing, West Sussex, BN14 8NW) placed in the centre of the combusting peat and was found to reach a maximum temperature of approximately 600 °C. However, a level of airflow was allowed to pass over the burning peat by placing it in a metal beaker which contained holes round its circumference about 3 cm above the peat sample (Fig 2.34). The airflow was determined by the flow rate of the fume hood under which the apparatus was placed and was maintained at approximately 0.88 m s^{-1}.

To simulate the set up found in an industrial kiln for the production of a typical peated malt, 50 g of dried peat was burned per 750 g of green malt. The initial peat moisture content was measured using the same protocol as used in Chapter 2.1.1 and the values for dried peat composites are shown in Table 2.25. In industry, the peat has a higher initial moisture content and water is often added throughout the peating process in an attempt to prevent flaming combustion. Therefore 25 mL UHQ water was mixed with each 50 g of peat.

Table 2.25. Moisture contents of dried peat composites.

Peat source	Composite moisture content (%)
Gartbreck	11.6
Glenmachrie	12.3
Castlehill	14.4
Orkney	11.1
St Fergus	13.9
Tomintoul	13.8

Malt temperature

As high temperatures denature the enzymes found in malt, cooling of the smoke prior to it contacting the malt was necessary. In industry, the temperature of malt is generally not allowed to exceed 60–65 °C when the malt is moist [10]. To try and achieve this in the lab-scale set up, two steel funnels were used to separate the malt bed from the peat burning vessel with the aim that as the peat smoke passed through the narrow passage connecting the two funnels heat would be lost conductively (Fig. 2.34). In this way, the temperature of the smoke as it contacted the malt was reduced, though it was still high enough to be potentially denaturing to malt enzymes (peaking at about 75 °C). However, attempting to separate the two metal funnels with a length of PVC tubing did not reduce the smoke temperature significantly (now peaking at about 69 °C). Therefore, it was decided to use the apparatus with the two funnels directly linked with perhaps some loss of enzymatic activity. To try and ensure that the airflow over the burning peat was even, a plastic guard was placed in front of the apparatus to disrupt the airflow through the fume hood.

Peat combustion time

Once a lab-scale peating system was in place it was necessary to determine a suitable peating time. As initial tests suggested that smoke was no longer produced after 30 min, this was selected as the peating time. To determine whether increasing the peating time would have an effect, a peating time of 45 min was compared. Peating was carried out using Tomintoul peat in triplicate. The peated and kilned samples were analysed using the previously developed HS-SPME method to quantify the marker phenols. Samples

were extracted in triplicate. ANOVA was then used to analyse the results (Table 4.26) and the mean concentrations are presented in Fig. 2.35.

Table 2.26. ANOVA for peating time of 30 mins versus 45 mins. P values are significance levels (below 0.05 indicates a significant difference).

Compound	P value
Guaiacol	0.0294
Methylguaiacol	0.0239
o-Cresol	0.0602
Phenol	0.0590
Ethylguaiacol	0.0087
p-Cresol	0.0425
m-Cresol	0.1618
4-Ethylphenol	0.0035

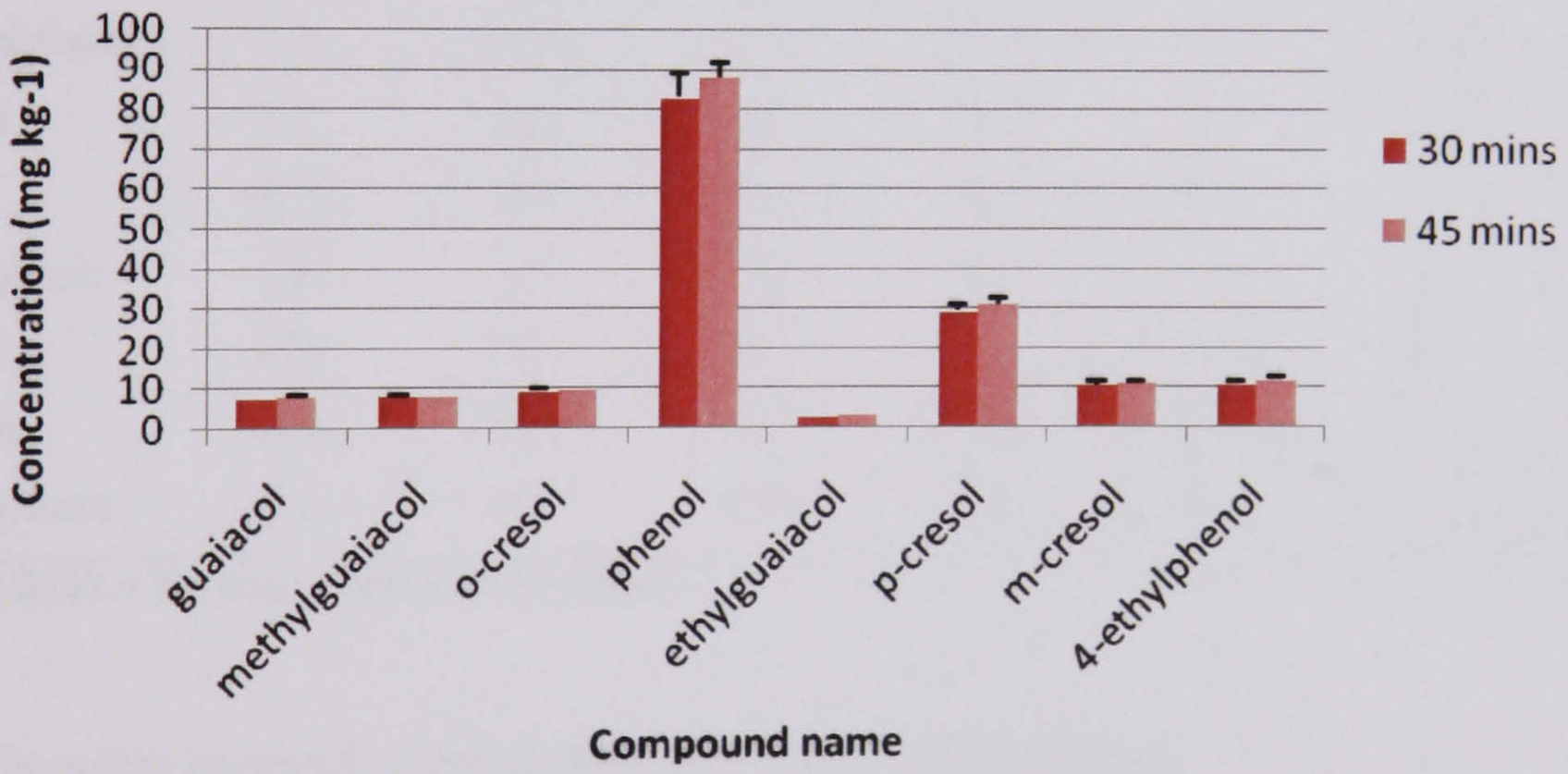


Figure 2.35. Effect of peating time on marker phenol concentrations in malt.

There was found to be a significant effect of changing the peating time for some compounds (guaiacol, methylguaiacol, ethylguaiacol, *p*-cresol and 4-ethylphenol). The 45 min peating yielding slightly higher concentrations of all the compounds tested (Fig. 2.35). However, the differences were small and so it was decided to carry out the peating process for 30 min as this would minimise the time the malt was exposed to potentially harmful high temperatures.

Reproducibility

As controls over the peat combustion were limited, it was important to determine how reproducible this process was. To assess the reproducibility, the concentrations of each of the eight marker phenols were measured in three peated malt samples produced using the same peat (Tomintoul). Each peated malt samples was analysed in triplicate. ANOVA was then used to determine if there was a significant difference between malt samples (Table 2.27). It was found that there was no significant difference between the peated malt samples. Therefore, the reproducibility of the method was satisfactory.

Table 2.27. ANOVA to determine reproducibility of peating method (a p value <0.05 indicates a significant difference between malts).

Compound	Malt a		Malt b		Malt c		p value
	Conc. (mg kg^{-1})	% RSD[a]	Conc. (mg kg^{-1})	% RSD[a]	Conc. (mg kg^{-1})	% RSD[a]	
Guaiacol	7.7	7.3	7.5	5.2	7.9	0.8	0.582
Methylguaiacol	7.7	7.0	7.7	6.9	8.1	3.1	0.526
o-Cresol	9.6	8.0	9.3	5.0	9.1	2.9	0.602
Phenol	84.3	10.1	85.8	6.9	79.0	2.8	0.419
Ethylguaiacol	2.8	5.7	2.8	9.4	2.9	4.3	0.594
p-Cresol	28.5	10.2	30.2	6.6	28.6	3.4	0.561
m-Cresol	10.9	10.0	11.1	5.1	10.1	3.0	0.295
4-Ethylphenol	10.6	8.6	11.4	6.8	11.0	5.0	0.453

[a] % RSD = Relative Standard Deviation.

Effect of peating method on predicted spirit yield (PSY) of malt

Once a reproducible method had been developed for the peating of malt, it was then necessary to determine if this malt was viable for the production of spirit. To do this, the PSY was measured in malt which had been subjected to the lab-scale peating process using Tomintoul peat. This value was then compared with that obtained from unpeated malt. The results from duplicate analyses (Table 2.28) demonstrated that the peating method did indeed have an effect on the PSY of the malt. It was decided however, that this effect was low enough to allow spirit production to go ahead using the peated malt.

Table 2.28. PSY (dry basis) of malt which has been peated compared with unpeated malt. Peated value is the average of three peated malts. Unpeated value is the average of two unpeated malt samples.

Unpeated		Peated		
PSY (litres ton^{-1})	**% RSD**	**PSY (litres ton^{-1})**	**% RSD**	**p value**
411.2	0.66	402.3	0.21	0.0000

Conclusions

Given the unpredictable nature of the combustion process, the main issue when trying to produce peated malt on a laboratory scale, was to develop a method for the reproducible combustion of peat. When using an enclosed furnace to burn peat, no smoke was produced. With very low airflow and a relatively slow heating rate, slow pyrolysis was probably responsible for production predominantly of char from peat as opposed to the desired smoke [111]. The high residence time of thermal breakdown products in the heated zone probably resulted in vigorous secondary reactions in which they were further pyrolysed to smaller, more highly combustible products [112]. In this way the production of particulate matter in the form of smoke was low. When the furnace door was opened, there was an immediate release of smoke suggesting that some level of airflow would be needed for the desired smoke formation.

The apparatus that was eventually used to produce the incomplete combustion of peat did not give the same temperature control as the furnace. Nevertheless the resulting peated malt composition was found to be reproducible. This method, therefore, was used to produce peated malts using peat from the six sampled peat deposits.

2.14 Lab-Scale New-Make Spirit Production

2.14.1 Mashing

A 128 g sample of malt was weighed into a milling beaker and milled using a Buhler Miag Universal Laboratory Disc Mill (Buhler GmbH, Braunschweig, Germany, Post fach 3369, D-38023) set at 0.2 mm gap width using a feeler gauge. The resultant grist was

immediately placed into a tared container and 125 g (± 0.001 g) weighed out. This process was repeated three times to obtain a total of 500 g of grist from each malt sample.

Each 125 g aliquot of grist was transferred as quantitatively as possible to a mashing beaker and place in a Lochner LB 8 Electronic mashing bath (Lochner Labor and Technik GmbH, Bayreuth, Germany, D-95445) at 65 °C. Distilled water was heated to 68 °C and 325 mL added to the grist in the mashing beaker, carefully rinsing any excess flour in the grist container. Each mash was stirred thoroughly using a glass rod to eliminate any lumps and covered with aluminium foil. The mash temperature was maintained at 65 °C for 1 h with regular stirring. The mash was then transferred to a polypropylene centrifuge bottle and spun at 1200 g (2000 rpm) for 6 min in a Sanyo MSE Mistral 3000E centrifuge (MSE (UK) Limited, London, UK, SE26 5AZ). The supernatant was decanted and filtered under vacuum through a Buchner filter fitted with an unbleached Classic Calico cotton filter (John Lewis plc., London, UK, SW1E 5NN) into a 2-L Buchner flask. The residual grains were then transferred to the filter funnel and filtered under vacuum to apparent dryness. A glass reagent bottle stopper was used to tamp down the bed and maintain the vacuum. The resulting filtrate was cooled to ambient temperature and transferred to a 1-L measuring cylinder. The volume of this first wort was noted (V1). The mashing efficiency was monitored very approximately by measuring the sugar content of a drop of wort using a pocket saccharimeter. (Calculation: Approx OG = % sugars x 4). The cooled first worts were place in a 3-L round bottomed flask and pitched with 8 g (0.36% (w/v)) pressed distillers 'M' type yeast and placed in a water bath at 19 °C.

The dried grains were returned to the mashing beaker and placed in the water bath which had been heated to 80 °C. The centrifuge bottle was rinsed with 200 mL boiling distilled water and the hot washings added to the grains as second water. The grains were extracted at 80 °C for 30 min, and then filtered under vacuum through the Buchner apparatus as described previously. The extracted second worts were cooled to ambient temperature and the volume (V2) recorded. The approximate OG of the second wort was

determined using a pocket saccharimeter. The cooled second wort was added to the fermentation flask.

The grains were returned to the mashing beaker and the beaker placed in a water bath at 100 °C and allowed to equilibrate for 5 min. Subsequently, 150 mL boiling water was added to the grains and extracted at 100 °C for 10 min. The grains were then filtered as quickly as possible in order to maximise elution of wort sugars before cooling. The extracted third worts were cooled and the volume (V3) measured. The approximate OG of the third worts was determined using a pocket saccharimeter. An appropriate volume of V3 was added to the wort in the fermenter in order to achieve a total wort volume of 2200 mL.

2.14.2 Fermentation

Once all the wort had been collected, the fermentation flask was sealed with a fermentation lock and placed in a water bath programmed from 19 °C to 33 °C in 3 days (66–72 h).

Wash alcohol strength

Subsequent to fermentation, 125 mL of wash was decanted into a 500-mL round bottomed flask and made up to 150 mL with distilled water. A few drops of silicone antifoam (10% solution of Sigma antifoam A concentrate (Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK, SP8 4XT)) were added along with 2–3 glass beads. The flask was attached to the distillation apparatus shown in Fig. 2.36. Distillation proceeded over a single Bunsen flame the distillate collected in a 100-mL 'A' grade volumetric flask. The flask containing the distillate was removed and stoppered after filling to just below the mark and allowed to equilibrate to 20 °C in a water bath for at least 20 min, then made up to the mark with distilled water. The spirit was mixed thoroughly and then filtered through a Whatmans GF/A (12.5 cm) filter paper (VWR International Ltd, Lutterworth, Leicestershire, UK, LE17 4XN), recycling the first 25–30 mL of filtrate. The density of

the filtrate was measured on the Anton Paar DMA 55 density meter (Anton Paar Scientific Ltd., Hertford, Herts, UK, SG13 7NW) and the alcohol content determined.

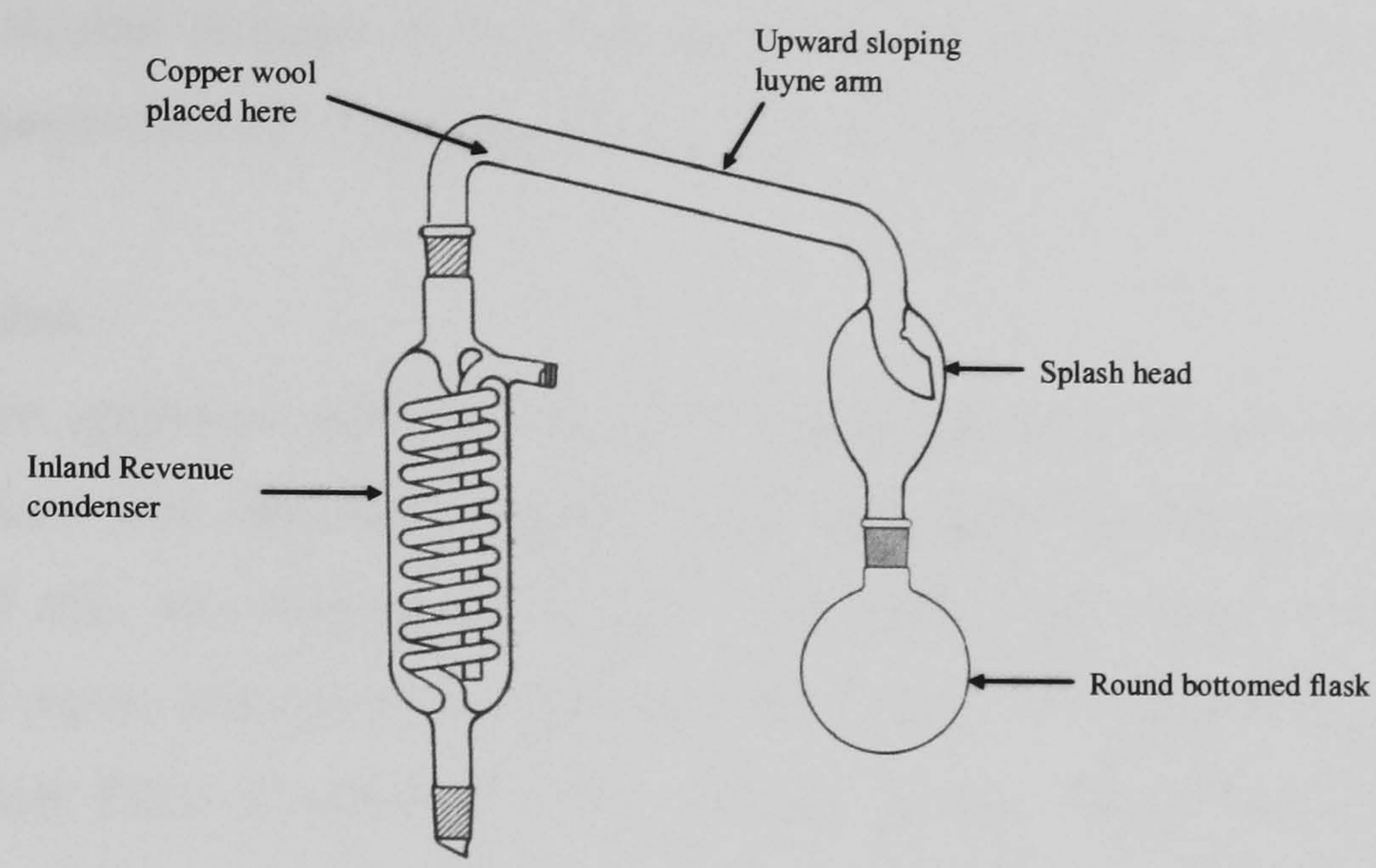


Figure 2.36. Distillation apparatus used for all lab-scale distillations (copper wool was not included for wash alcohol strength distillations).

Final gravity

Seventy five millilitres of wash was decanted into a 32-cm diameter fluted filter paper (Whatman 54 (VWR International Ltd, Lutterworth, Leicestershire, UK, LE17 4XN) or Ederol 12 (H Rudebeck & Company, Burgess Hill, West Sussex, UK, RH15 9LH)) and the filtrate collected. The first 10–20 mL of wash was returned to the filter. The density of the filtrate was measured using the DMA 55 and used to calculate the FG.

2.14.3 Distillation

Wash distillation

Two or three glass beads were added to 2000 mL of wash and the flask was attached to the the distillation apparatus shown in Fig. 2.36. Ten grams of copper wire (Trollull Ltd., Andover, Hants., UK, SP10 5AZ) was placed at the top of the lyne arm. Heat was supplied using a single Bunsen burner at maximum flame height. When the distillate appeared at the foot of the condenser, the time was noted as the **Still-in** time. The distillate/ low wines (LW) were collected (750 mL) in a measuring cylinder (500 mL)

and covered with aluminium foil to minimise the loss of volatiles. Seven hundred and fifty millilitres of LW were collected over about 3 h. When the required volume of LW had been collected the **Still-off** time was noted. The LW was mixed thoroughly and the approximate alcohol strength of the LW measured using the DMA 55 density meter (Anton Paar Scientific Ltd., Hertford, Herts, UK, SG13 7NW).

Spirit distillation

The distillation apparatus used for the wash distillation was cleaned and used for the spirit distillation. Ten grams of copper wire was again placed at the top of the lyne arm. The LW (700 mL) was charged to the still. The starting time was noted and heat was applied at full power until the top of the still neck was hot. The heat was cut back to give a half full height flame and the LW allowed to boil gently. When the distillate began to drop into the receiver, the time was noted as the **Still-in** or **On-spirit time.** Five millilitres of foreshots was collected in a 10-mL measuring cylinder and subsequently 155 mL spirit was collected in a 200-mL measuring cylinder. These fractions were collected over a period of about 70 min. The distillation rate was maintained by adjusting the height of the Bunsen burner. The **Off-spirit** time was noted. Once the spirit was collected, the feints (235 mL) were collected. The heat was increased so that the feints fraction was collected over approximately 60–70 minutes. Once all the feints were collected the **Still-off** time was noted. The approximate alcohol strengths of the spirit and feints were measured using the density meter.

2.14.4 Method development

Though the production of lab-scale new-make spirits was carried out in accordance with previously developed methods (SWRI), initial experiments highlighted some factors that needed to be optimised.

Mashing filter

As stated previously, after each of the three mashing stages (65 °C, 80 °C and 100 °C), the wort was removed from the residual grains by filtration under vacuum thus yielding

three wort fractions (V1, V2, and V3). Initially, this process was carried out using one of two separate electric vacuum pumps: pump a (Millipore (U.K.) Ltd., Watford, UK, Herts, WD18 8YH) or pump b (KNF Neuberger U.K. Ltd., Witney, Oxon, UK, OX28 4FA). It was found that using the two different pumps had a significant effect on the volume of wort extracted (Table 2.29).

Table 2.20. Effect of filter pump on wort volume.

Pump	Cases	V1			V2			V3		
		Mean	Standard Deviation	p value	Mean	Standard Deviation	p value	Mean	Standard Deviation	p value
a	6	915.83	25.96	0.0000	715.83	18.00	0.0115	605.83	68.29	0.0020
b	6	1050.83	20.10		741.67	9.83		485.00	21.45	

Table 2.29 shows that pump b was more efficient than pump a. In order to obtain the same total wort volume for fermentation using the two pumps it was necessary to add an excess of water to the third mashing stage when using pump a, hence the higher V3 volume for this pump. It was subsequently found that this variation in the mashing profile had an effect on the final spirit composition (Fig. 2.37).

Figure 2.37 shows that using the less efficient pump resulted in a higher concentration of marker phenols. The concentration of the marker phenols was found to be inversely related to the gravity and alcohol strength. It was also noted that pump gave a more variable results than pump b.

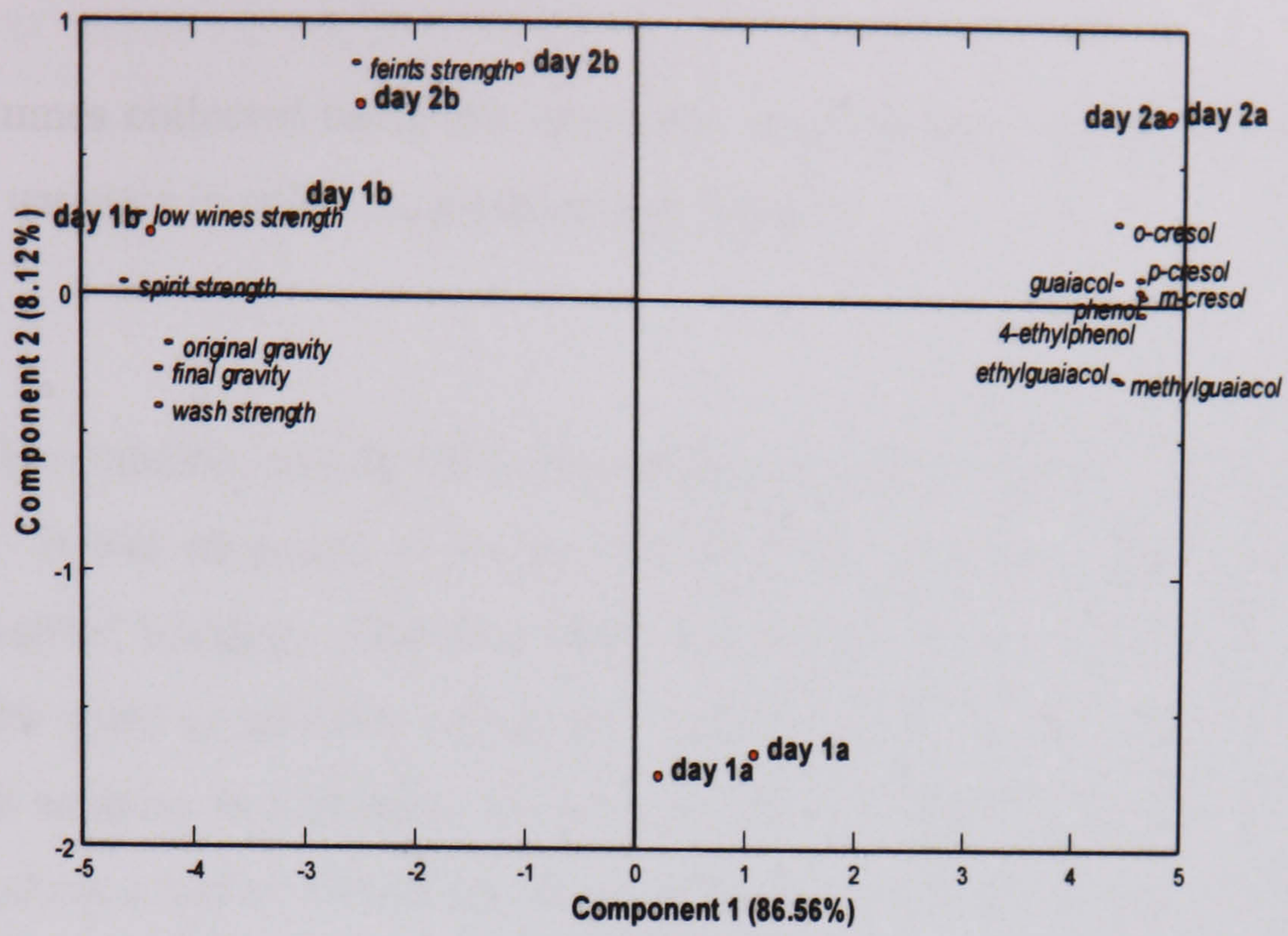


Figure 2.37. PCA biplot of the spirits made using pumps a and b on two consecutive days (data from duplicate analysis of each sample shown). The marker phenol concentrations, sample strengths and gravities are loadings.

Therefore, to produce a reproducible spirit, it was important to use the same filtration apparatus during the mashing procedure. Using the filtration set up shown in Fig. 2.38 it was possible to filter two samples simultaneously using a single filter pump (pump b).

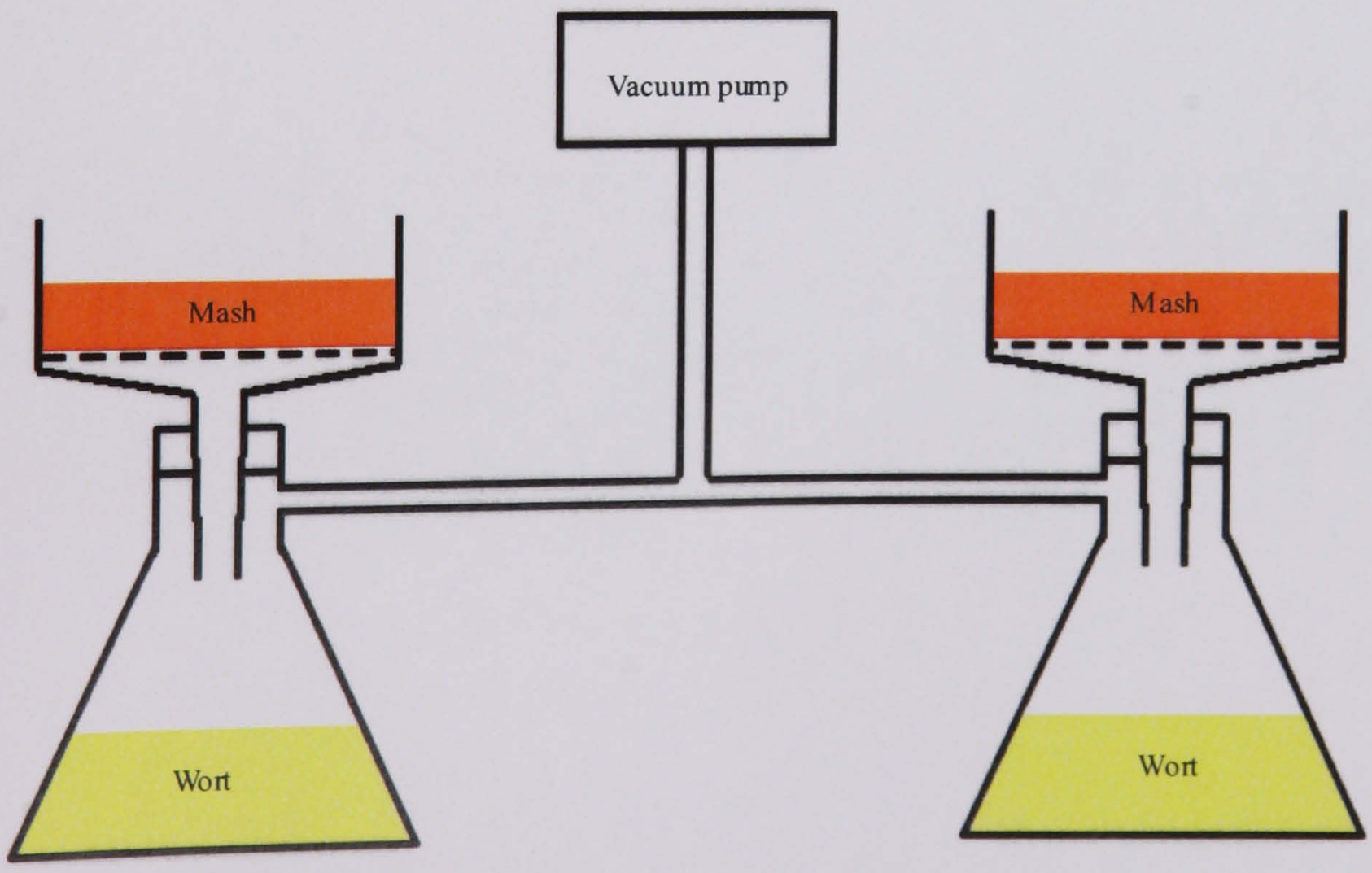


Figure 2.38. Mash filter system utilised to ensure reproducible filtration of samples.

The wort volumes collected using this apparatus was found to be more reproducible and therefore the variance in spirit composition was reduced.

Yeast

In terms of fermentation, one factor to be looked at was the effect of yeast on the spirit composition. It was proposed to use pressed 'M' type distillers yeast and this type of yeast has a limited lifespan. Therefore different batches of yeast would have to be used throughout the spirit production schedule. There were six peated malts to be used to produce spirit samples in triplicate. So that any effect of using different yeast batches on spirit composition could be identified and eliminated by statistical analysis, each replicate set of peated malt samples were to be fermented using a single batch of yeast. Therefore, three batches of yeast were to be used and each batch was to be used for six fermentations (Table 2.30).

Table 2.30. Schedule for lab-scale new-make spirit production.

Day	Samples for mashing and fermentation		Samples for distillation		Yeast batch
day 1	St Fergus	Castlehill			
day 2	Hobbister	Tomintoul			
day 3					
day 4			St Fergus	Castlehill	1
day 5			Hobbister	Tomintoul	
day 6					
day 7					
day 8	Gartbreck	Glenmachrie			
day 9	Hobbister	Castlehill			
day 10					
day 11			Gartbreck	Glenmachrie	
day 12			Hobbister	Castlehill	2
day 13					
day 14					
day 15	Gartbreck	Tomintoul			
day 16	Glenmachrie	St Fergus			
day 17					
day 18			Gartbreck	Tomintoul	
day 19			Glenmachrie	St Fergus	
day 20					
day 21					
day 22	Glenmachrie	Castlehill			
day 23	Tomintoul	St Fergus			
day 24					3
day 25			Glenmachrie	Castlehill	
day 26	Hobbister	Gartbreck	Tomintoul	St Fergus	
day 27					
day 28					
day 29			Hobbister	Gartbreck	

From the data in Table 2.30 it was calculated that each batch of yeast would have to be kept for up to eight days to allow the processing of six peated malt samples. Therefore it

was important to test whether the character of the spirit produced over this time period was in any way altered due to changes in the yeast performance. As such, spirit was produced over this time period using a single batch of yeast and a single batch of unpeated wort. The spirit was then checked for consistency of character by sensory analysis using Triangle Tests (for method see Chapter 2.12.3). The results shown in Table 2.31 showed that the panel could not distinguish between spirits produced using the same batch of yeast over this time period.

Table 2.31. Triangle tests performed on 2 spirits produced on day 1 (A and B) compared with those produced on day 8 (A and B) using the same yeast batch.

Comparison	Number of correct responses (out of 20)	Significance
day 1 A v day 8 A	6	No significant difference
day 1 B v day 8 B	5	No significant difference

Method reproducibility

Using the previously described methods, spirit samples were produced in triplicate using the six lab-scale peated malts. To determine how reproducible these samples were, they were analysed initially by sensory analysis using Quantitative Descriptive Analysis (for method see Chapter 2.12.4). Results are shown in Table 2.32.

Table 2.32. P values for ANOVA carried out on Quantitative Descriptive Analysis data for replicates of six peated new-make spirit. Significant values are coloured red.

Sample name	Intensity of peaty character	Burnt	Smoky	Medicinal	Other new-make characteristics
St Fergus	0.5816	0.3954	0.8011	0.2563	0.5951
Castlehill	0.5112	0.2943	0.4361	0.5008	0.6024
Tomintoul	0.2232	0.0813	0.1821	0.2298	0.3737
Hobbister	0.8806	0.1048	0.0579	0.2736	0.2477
Glenmachrie	0.4212	0.8668	0.7127	0.0250	0.4009
Gartbreck	0.5304	0.4080	0.0034	0.7458	0.0443

Only minor aroma differences were observed between replicate samples. These were for the medicinal attribute in Glenmachrie samples and for the smoky and other new-make characteristics in the Gartbreck samples.

To assess the analytical reproducibility of the new-make spirit samples, they were analysed for the eight marker phenols using GC-MS. Table 2.33 shows that, with one exception, the % RSD for all compounds and all peat types was below 8%.

Table 2.33. Concentrations of marker phenols in lab-scale new-make spirit. Triplicate samples for each peat type were analysed in duplicate. Concentrations are given in ppm (values in brackets are % RSD).

Compound	Gartbreck	Hobbister	Glenmachrie	St Fergus	Castlehill	Tomintoul
Guaiacol	7.9 (7.2)	4.9 (2.3)	9.9 (4.5)	7.7 (5.8)	10.0 (2.5)	4.8 (3.6)
Methylguaiacol	4.7 (6.1)	3.1 (3.5)	6.4 (7.4)	6.0 (6.5)	6.5 (3.9)	3.7 (9.0)
o-Cresol	4.2 (6.0)	3.2 (2.9)	4.7 (4.7)	4.0 (4.0)	4.6 (2.8)	4.0 (2.1)
Phenol	6.7 (6.1)	4.8 (2.3)	8.4 (5.3)	6.1 (3.9)	7.2 (2.8)	6.8 (6.7)
Ethylguaiacol	2.9 (5.6)	2.0 (2.8)	3.9 (6.9)	3.2 (5.5)	4.1 (3.8)	2.3 (7.1)
p-Cresol	3.7 (5.4)	2.5 (2.0)	4.7 (5.1)	3.5 (2.3)	4.4 (2.6)	3.6 (5.7)
m-Cresol	1.7 (4.6)	1.3 (1.9)	1.6 (5.0)	2.0 (2.0)	1.9 (2.3)	1.8 (5.8)
4-Ethylphenol	2.0 (4.8)	1.5 (1.4)	2.4 (6.0)	2.0 (3.3)	2.5 (3.3)	1.9 (5.0)
Total phenols	33.8 (4.7)	23.4 (1.6)	42.0 (5.4)	34.5 (4.2)	41.1 (2.7)	28.8 (5.0)

Conclusions

With some minor optimisation it was found that the existing method for production of spirit on a laboratory scale could be used to produce reproducible peated new-make spirit. The minor variations detected in spirit aroma were addressed by making composites of replicate samples and using these to compare spirit produced using different peat.

2.15 Statistical Analysis

2.15.1 Analysis of variance (ANOVA)

The purpose of analysis of variance is to test differences in group means for statistical significance [113]. This is accomplished by analysing the variance, where variance is computed as the sum of squared deviations from the overall mean, divided by $n - 1$ (sample size minus one). The total variance is partitioned into the component that is due to true random error (i.e. within-group variability) and the components that are due to differences between means (i.e. between-group variability). These latter variance components are then tested for statistical significance. This is achieved by a comparison of the variance due to the between-groups variability (called *Mean Square Effect*, or MS_{effect}) with the within-group variability (called *Mean Square Error*, or Ms_{error}). These two estimates of variance are compared via the F test, which tests whether the ratio of the two variance estimates is significantly greater than 1. If significant, the null hypothesis of no differences between means is rejected, and the alternative hypothesis that the means (in the population) are different from each other is accepted.

The variables that are measured are called *dependent* variables. The variables that are manipulated or controlled are called *factors* or *independent* variables. Inclusion of more than one factor can minimise within group variance and thus increase the sensitivity (power) of a test. ANOVA also allows the detection of *interaction* effects between variables. Interactions are the extent to which the effects of one factor differ according to the levels of another factor.

ANOVA were performed using Unistat statistical software (version 5.0) (Unistat Ltd, London, UK, W9 3DY).

2.15.2 Principal component analysis (PCA)

PCA is one of the simplest examples of a multivariate statistical method. It is an example of an unsupervised or exploratory method. The object of PCA is to take *p* variables X_1, X_2,..., X_p and find combinations of these to produce indices Z_1, Z_2,..., Z_p that are uncorrelated. In this way, PCA can be used for reducing the dimensionality of the original data without decreasing their variance [114]. The indices, or principal components, are ordered such that Z_1 displays the largest amount of variation, Z_2 displays the second largest amount of variation and so on. It is normally desired that most of the variation in the data set can be described by the first few principal components and the remaining components, with very low variances, can reasonably be ignored. A scatter plot can then be drawn to visualise the results. Distribution of the samples and the variables on this plot allows interpretation of the major differences among samples.

PCA was performed using Unistat statistical software (version 5.0) (Unistat Ltd, London, UK, W9 3DY).

2.15.3 Discriminant function analysis (DFA)

DFA (also known as canonical variates analysis (CVA)) is a supervised projection method [115]. *A priori* information about sample grouping is used to produce measures of within-group variance and between-group variance. This information is then used to define discriminant functions which optimally separate the *a priori* groups. These functions can then be used as a co-ordinate system to visualise the DFA scores. This method was used to investigate FT-IR fingerprint spectra's potential for discriminating between peat geographic locations, and subsequently observing the inter-group pattern of clustering, to establish the extent to which the patterns relate to the geographical location of the sample peat.

DFA cannot be performed on the raw absorbance data set due to mathematical instability. The starting point for DFA is the inverse of the pooled variance-covariance matrix within

a-priori groups. This inverse can only exist when the matrix is non-singular, i.e. its determinant is other than zero, which implies that it is of full rank [115,116]. Generally DFA can only be performed if:

$$N_s - N_g - 1 > N_v \quad (8)$$

Where, N_s is the number of samples, N_g is the number of a priori groups, and N_v is the number of variables. With FT-IR data, which typically has ~1600 variables, direct DFA is not possible. One solution is to perform PCA as a pre-processing step. PCA is a well known technique for reducing the dimensionality of multivariate data whilst preserving most of the variance [41].

As the two-stage PCA-DFA is a supervised projection method, some strategy is needed to pick the optimal number of principal components to project into DFA space. The only robust way of estimating the correct number of PCs is by carrying out some sort of cross validation, in this case training set / test set validation. This strategy involves simply performing a linear search. Starting with *m* PC scores (where, *m* is determined by manipulating equation 1.0) a DFA model is built using the training set and validated using the test set. The number of PCs is then reduced by 1 and another DFA model is built and validated. This process is repeated until a set of *m* DFA models have been built. The optimal number of PCs is then determined visually, or statistically, by looking at the DFA scores for each model in turn. The optimal model is the one that provides maximal group separation such that 'test set' members of a given group are correctly classified.

Finally, the Euclidean distance between *a priori* group centres in PC-DFA space was used to construct a similarity measure, with the Gower general similarity coefficient S_G, and these distance measures were then processed by an agglomerative clustering algorithm to construct a dendrogram [117].

In this study all models were built using the Matlab interactive scientific programming environment (The Mathworks Inc., Natick, MA 01760-2098, USA). PCA was

implemented using the NIPALS algorithm [118]. DFA was implemented as CVA; with the scores normalised to unit within-group variance allowing 95% χ^2 confidence regions to be plotted around each *a-priori* class centre [119]. All Matlab scripts are available upon request.

Chapter 3: Results

3.1 Peat Composition

3.1.1 Introduction

The aim of this work was to determine if there were any chemical differences in peat sampled from the various locations around Scotland used by the Scotch whisky industry. The first objective was to use a high throughput fingerprinting technique, FT-IR in combination with multivariate statistical analysis, to screen a large number of peat samples from various locations and determine whether there was a difference due to location. Subsequently, a chemical profiling technique, Curie point pyrolysis in combination with GC-MS, was used to identify the compounds responsible for differentiating peats.

3.1.2 Differentiation of peat from different geographical locations using FT-IR

FT-IR spectra

Typical FT-IR spectra produced from peat from the six different locations are shown in Fig. 3.1. Major peaks associated with fatty acids, peptides and carbohydrates can be readily observed as detailed in e.g. [120]; however, all these spectra (and indeed the others collected) showed similar broad features with very few visibly discernible qualitative or quantitative differences between them. This illustrates the need to employ multivariate statistical techniques for the analysis of these FT-IR data. Given the complexity of these spectra it is usual that discriminant analysis algorithms are applied so that samples may be classified according to any groups detected in the data.

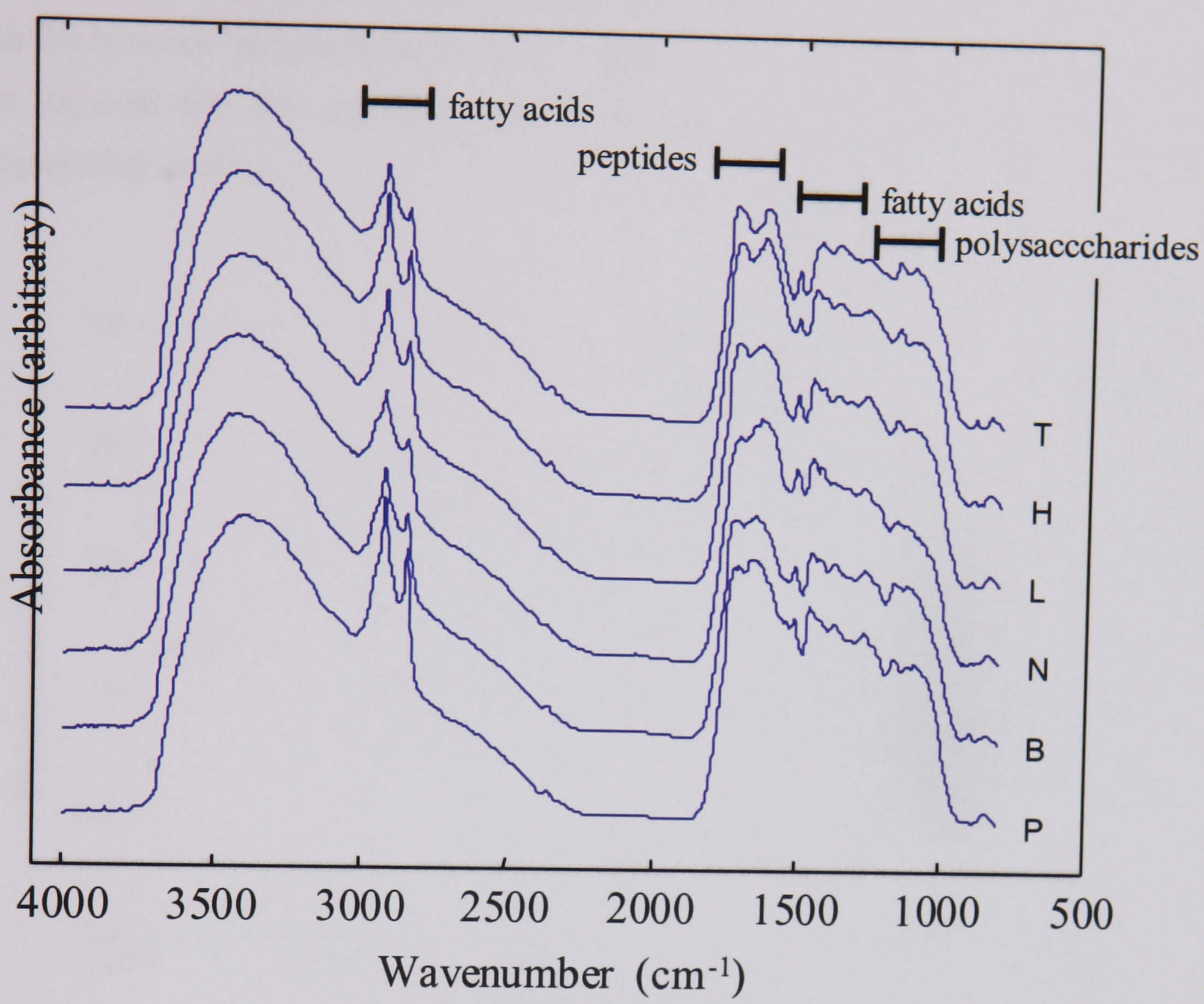


Figure 3.1. Typical FT-IR spectra from peat samples from the six different locations, encoded T, H, L, N, B and P (see Table 2.1 for details). These spectra are offset so that any differences can be more readily observed.

Cluster analysis of FT-IR spectra

The spectra were analysed using PC-DFA as described in Chapter 2.15.3. Initially the *a priori* knowledge of which spectra were replicates was used as the supervised class structure thus attempting to remove any analytical variance in the data without forcing the data to conform to some other more constrained classification (in this case geographical location) (Fig. 3.2). It can be seen in Fig. 3.2 that some samples were atypical, separating out to the left of the bulk of the peat samples. These atypical samples were all from St Fergus and in some cases the physical appearance (e.g. sample Nt15) suggested a high mineral soil content in place of peat. While by eye, peat samples from

Tomintoul tended to cluster towards the top of the plot, there was no clear separation of samples suggesting that the within 'geographical location' variability is more significant than the between 'geographical location' variability. From the initial PC-DFA results it was apparent that this approach was inadequate for discriminating peat samples by geographical location.

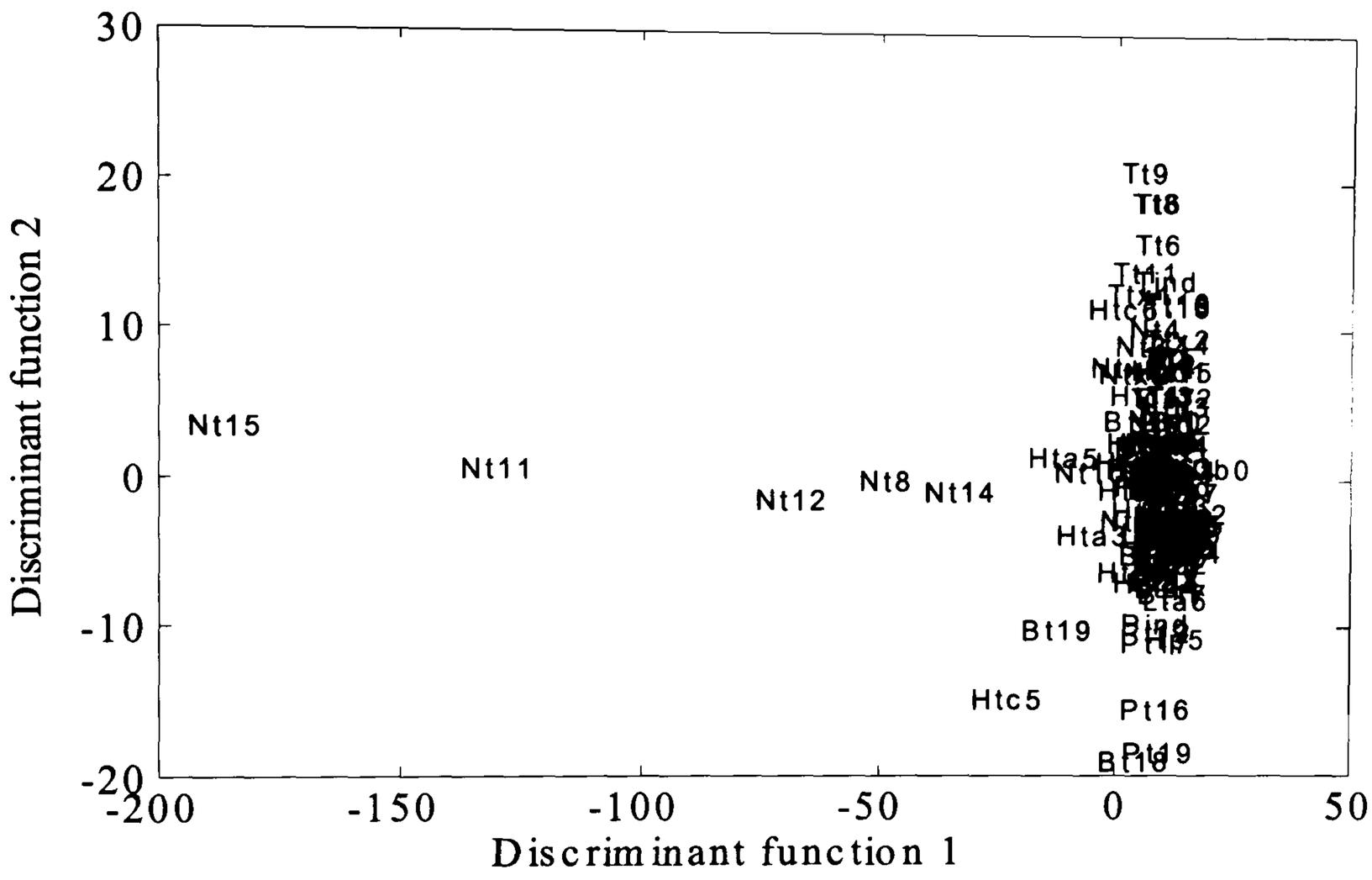


Figure 3.2. PC-DFA plot of all peat samples analysed. Samples were assigned individual classes in DFA, and PCs 1–50 used as input to DFA. The means of the nine sample replicates are shown. Sample codes are defined in Chapter 2.1.1.

In order to differentiate the peat types according to geographic location, this class structure was therefore assigned to the PC-DFA analysis; thus minimising both analytical variance and within location variance. Taking this approach, peats from two locations, St Fergus (N) and Tomintoul (T), could be clearly separated from the others in the first two PC-DF scores (Fig. 3.3).

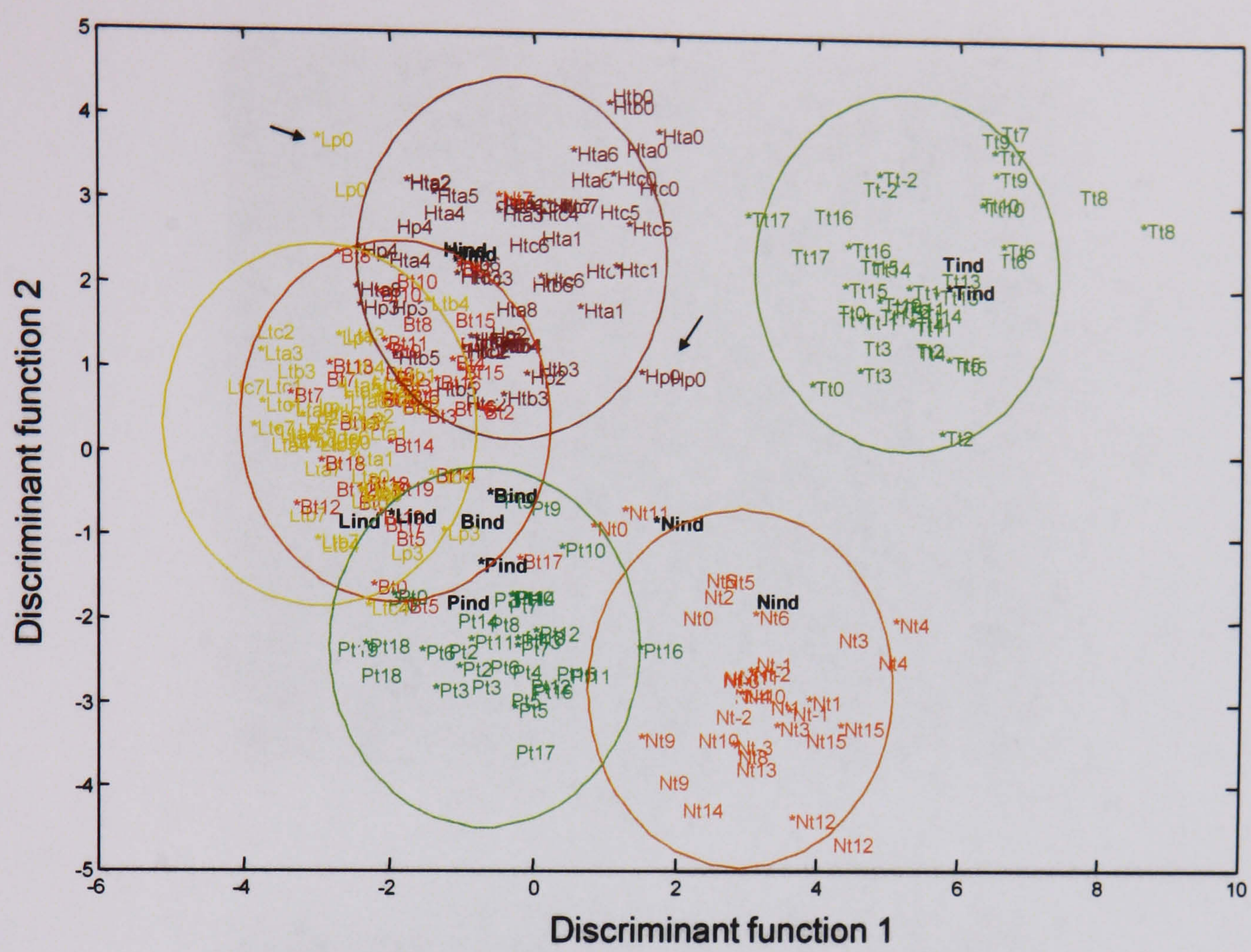
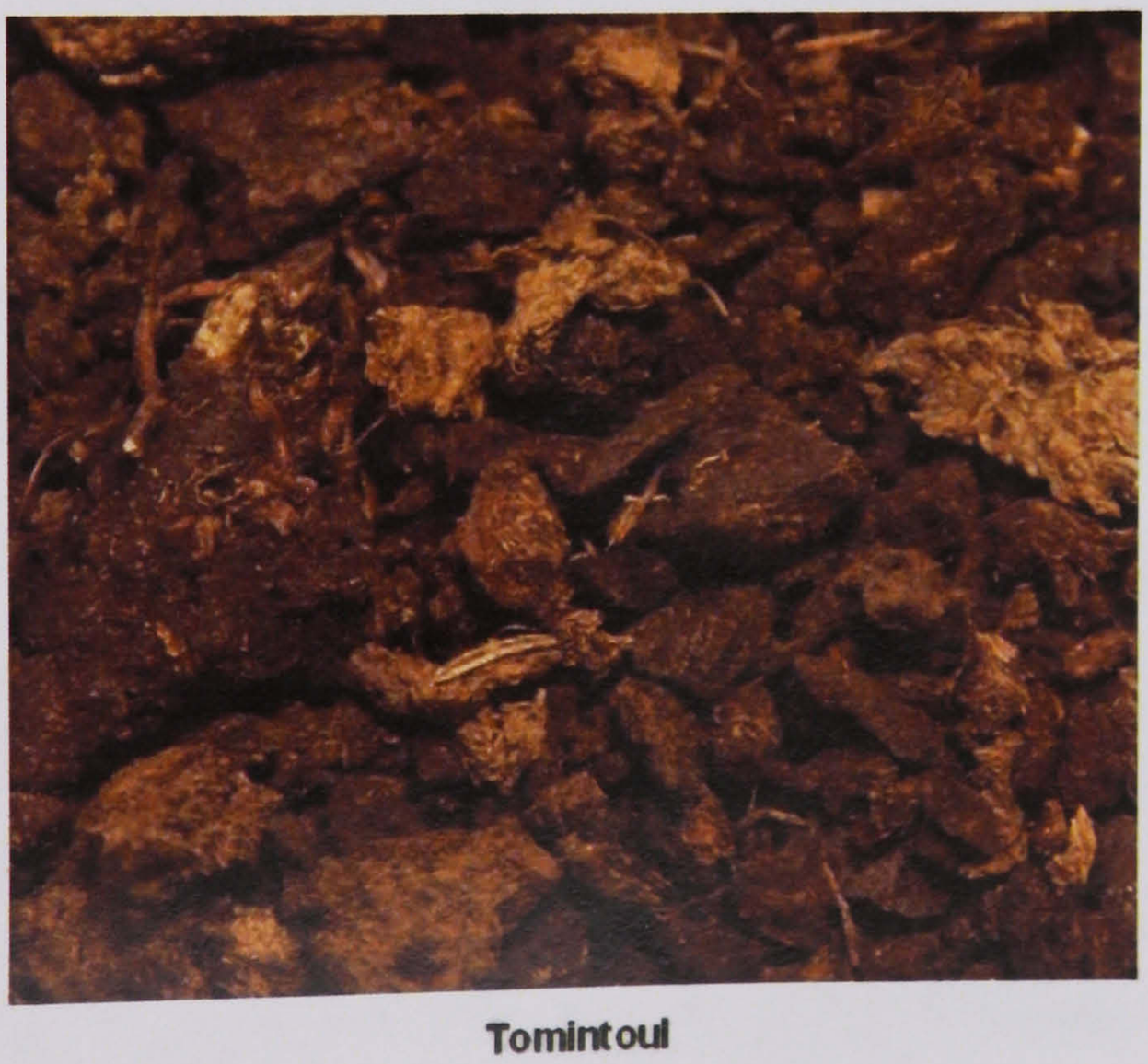


Figure 3.3. PC-DFA plot of all the peat samples encoded with knowledge of the six geographical locations from which the peat was collected. Training sets are denoted by symbols (e.g., Nt4) and the test set by symbols and asterisks (e.g. *Nt4). Circles represent 95% χ^2 confidence limits. Arrows represent outlier samples that were from the upper horizon of the peat depth profile.

On inspection, there were found to be differences in the physical appearance of samples taken from these two locations (Fig. 3.4). Of particular interest was the presence of woody remains in several of the St Fergus samples whereas in the Tomintoul samples the levels of recognisable woody material were low. Conversely, some of the samples taken from Tomintoul appeared to contain *Sphagnum* moss remains.

St Fergus

a



Tomintoul

b

Figure 3.4. Composite of line transect samples collected from St Fergus (a) and Tomintoul (b).

Whilst the other peats appear to overlap in the plot shown in Fig. 3.3, when the other PC-DF scores were inspected (data not shown) all peats were significantly different at the 95% confidence level except for Gartbreck moss (B) and Glenmachrie moss (L) from Islay which were statistically very similar and could not be distinguished. A dendrogram of the group means was constructed from these data which allowed a more quantitative assessment of the overall relative similarities between these peats to be visualised (Fig. 3.5). The dendrogram in Fig. 3.5 highlights the similarity among the peats from the three Islay locations.

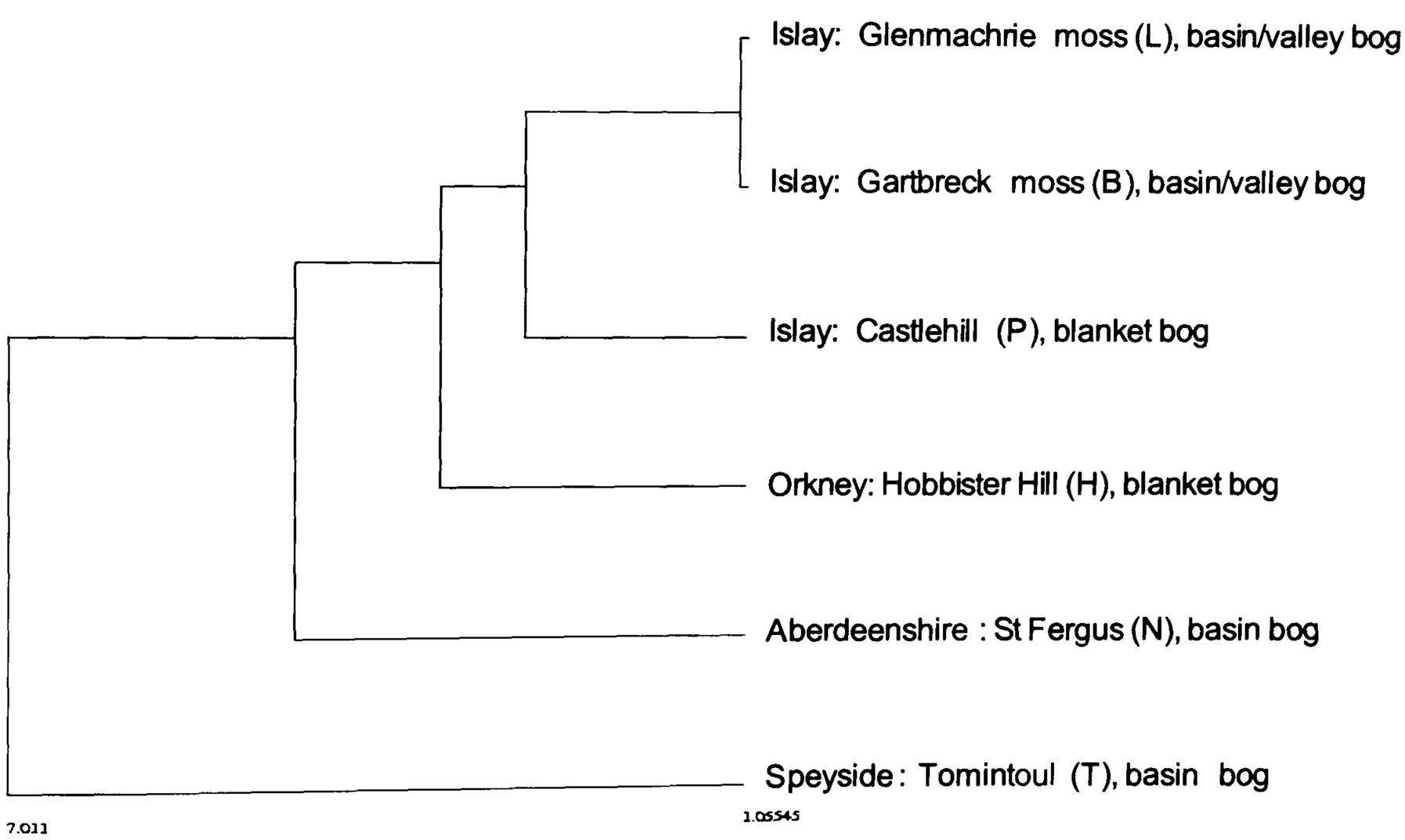


Figure 3.5. Dendrogram constructed from the PC-DFA group means showing the relationship between the six peat samples.

To assess the effect of cutting depth on peat composition, samples were taken from different depths at Glenmachrie and Hobbister Hill- the two locations where peat is extracted along banks. In both cases the samples taken from the upper horizon of the depth profile, Lp0 and Hp0 respectively were located outside the 95% confidence intervals (indicated by arrows) and so were distinct on the PC-DFA plot from the other

samples taken from these locations (Fig. 3.3). The remaining samples from each depth profile (with the exception of Hp1 which did not produce any useable spectral data) co-localised with the transect samples from their respective locations demonstrating that peat cut from different depths was, in general, chemically similar to the transect samples.

Finally, samples were taken from stocks of previously cut peat at each location to see how their composition related to that of the transect line samples. These samples were used in the PC-DFA shown in Fig. 3.3 and all co-localised with the transect samples from their respective locations demonstrating that industrially cut peat shared similar chemical characteristics with the transect samples and that peat samples could be identified by FT-IR to a principal location.

3.1.3 Chemical characterisation of peat samples from different geographical locations using Py-GC-MS

The FT-IR analysis showed that peat samples differed according to geographical location but this approach does not indicate differences in chemical structure. Samples were therefore analysed by Py-GC-MS in order to understand the chemical differences. Three samples were selected from each of the clusters corresponding to geographical locations identified in the FT-IR analysis: Hobbister Hill, Islay (due to the relative similarity of samples from the three Islay locations, Islay was treated as a single location for this analysis), St Fergus and Tomintoul. The samples were selected on the basis of their distinction from other sample clusters on the PC-DFA plot shown in Fig. 3.3 and are listed below (Table 3.1).

Table 3.1. Samples selected for Py-GC-MS analysis.

Geographical location	Sample code
Islay	Bt5
	Lta4
	Pt5
Hobbister Hill	Hta4
	Htb0
	Htc0
St Fergus	Nt1
	Nt10
	Nt12
Tomintoul	Tt6
	Tt10
	Tt14

A total ion current chromatogram (pyrogram) of the Py-GC-MS data of a typical peat sample is shown in Fig. 3.6.

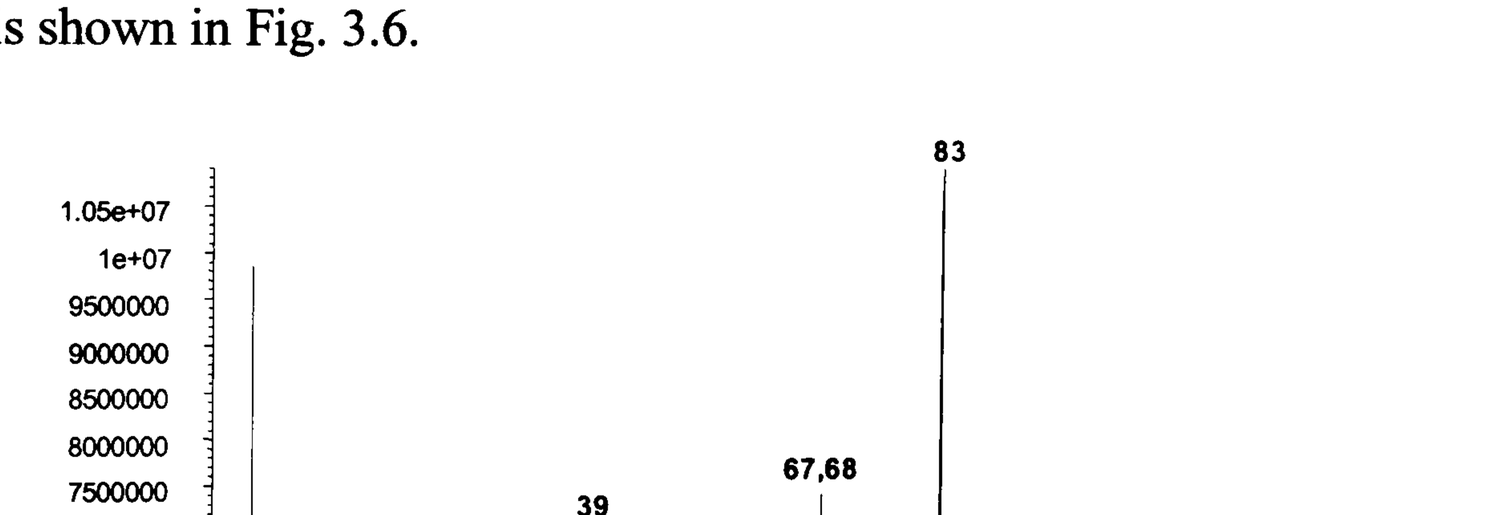


Figure 3.6. Total ion current chromatogram of pyrolysis products of peat sample Bt5. Identities of numbered peaks can be found in Table 2.6.

In total, 122 pyrolysis products were identified in the peat samples (Table 2.6 (Appendix A)). These were broadly split into the following classes: phenols, carbohydrate derivatives, aromatic compounds and nitrogen-containing compounds. The phenols were further divided into specific lignin markers (guaiacols and syringols) and non-specific phenolic compounds (henceforth referred to as phenols) such as catechols and phenols themselves. The aromatic compounds refer to those compounds of an aromatic nature which could not be included in one of the other classes. Some compounds detected in the pyrogram were omitted from the analysis either because they did not yield reproducible results or were not considered important for the production of peaty or smoky aromas. These included the poorly resolved anhydrosugars and also lipid-derived fatty acids.

Following Py-GC-MS analysis of samples from the four geographical locations, the data for the 122 identified compounds were normalised by expressing each peak area as a percentage of the total peak area. The normalised data were analysed by PCA. This analysis allowed the peat sources to be separated according to geographical location (data not shown). To eliminate redundant data and improve separation, an ANOVA was carried out on all the compounds analysed to see which were having a significant effect on the separation of the four geographical locations. In this way, the number of compounds was reduced from 122 to 111 whilst retaining representative compounds from the major classes described previously. These significant compounds were again normalised by expressing each peak area as a percentage of the total significant compound peak area (Table 3.2 (Appendix C)). PCA was then repeated using the 111 significant compounds.

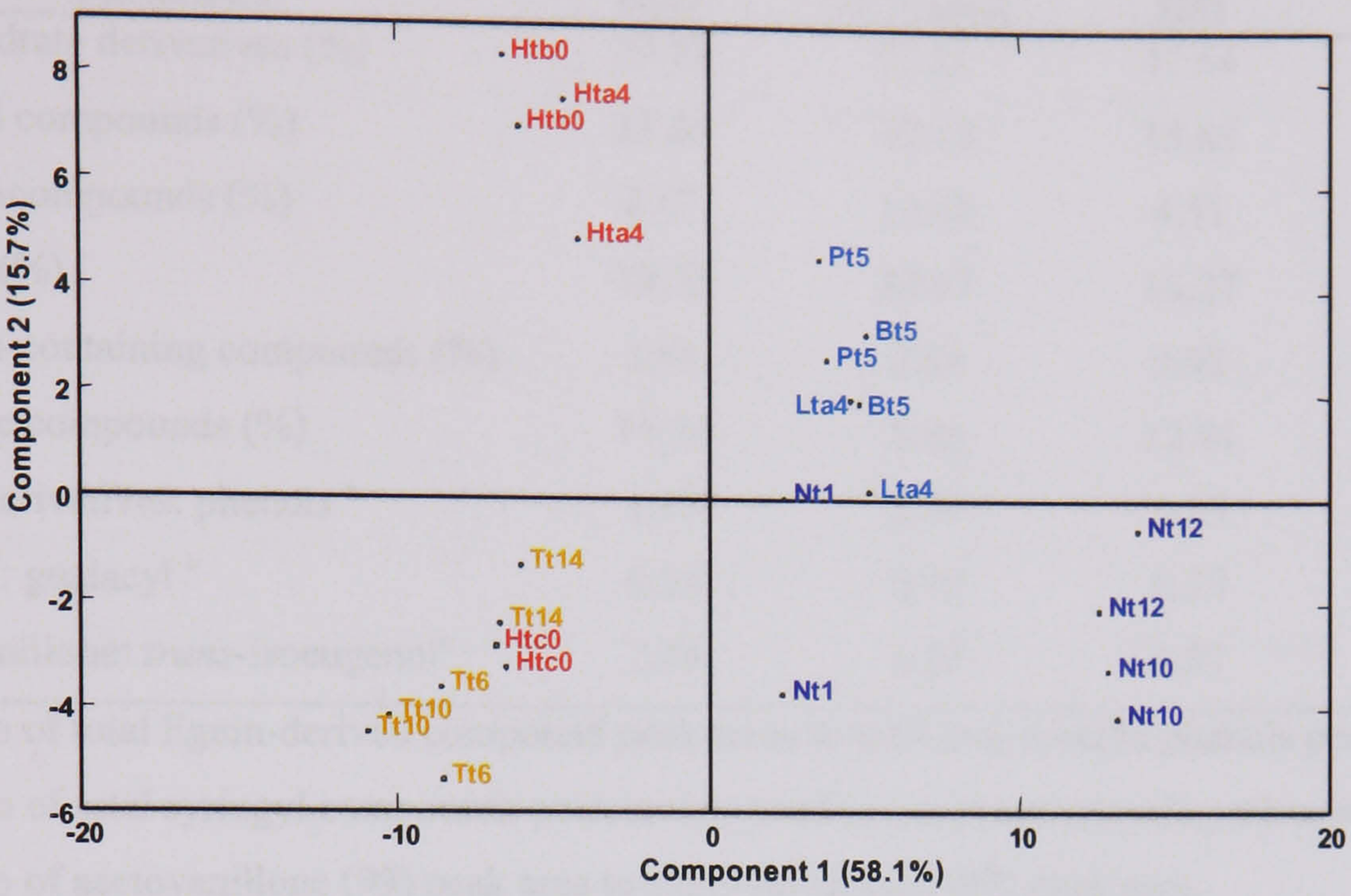
When analysing PCA data, only those principal components with an eigen value of 1 or more were considered (Table 3.3). This was because the variance represented by principal components with an eigen value of less than 1 was negligable. Using the four geographic locations as a factor, ANOVA carried out on the principal component values showed that the PCA model produced three components, explaining 83.95% of variance, which significantly differentiated peat samples from the four locations (Table 3.3).

Table 3.3. Variance table for PCA of Py-GC-MS data. P values are for ANOVA carried out on PC values using geographical location as a factor.

Component no.	Eigen value	Percent variance	Cumulative variance	p value
1	64.49	58.10	58.10	0.0000
2	17.39	15.67	73.76	0.0010
3	11.31	10.19	83.95	0.0000
4	3.77	3.40	87.35	0.2820
5	2.88	2.60	89.94	0.1450
6	2.33	2.10	92.05	0.6950
7	1.90	1.71	93.76	0.7810
8	1.25	1.12	94.88	0.9040
9	1.17	1.05	95.93	0.9490

The data for the first two principal components are plotted in Fig. 3.7. By examining the PCA loadings for this plot, it was found that the first principal component, which described 58.1% of the variance, mainly separated areas according to the ratio of carbohydrate derivatives to guaiacols, syringols and phenols (Fig. 3.7b and Table 3.4). St Fergus and Islay samples, located on the positive side of PC 1, were characterised by high percentages of guaiacols, syringols and phenols. Tomintoul and Hobbister Hill samples, located on the negative side of PC 1, were characterised by relatively high proportions of carbohydrate derivatives. Furthermore, the samples from Tomintoul were found to contain significantly higher proportions of carbohydrate derivatives than the samples collected from Hobbister Hill.

Unlike the specific, lignin-derived phenolic compounds (guaiacols and syringols), the other non-specific phenolic compounds were slightly less well clustered. Whilst the majority of these compounds co-localised with the lignin-derived phenols, three were found to locate to the negative side of PC 1 (phenol (**39**), 2-ethylphenol (**56**) and 4-acetylphenol (**95**)). Phenol and 2-ethylphenol were characteristic of the Hobbister Hill peat samples whilst 4-acetylphenol characterised the Tomintoul samples.



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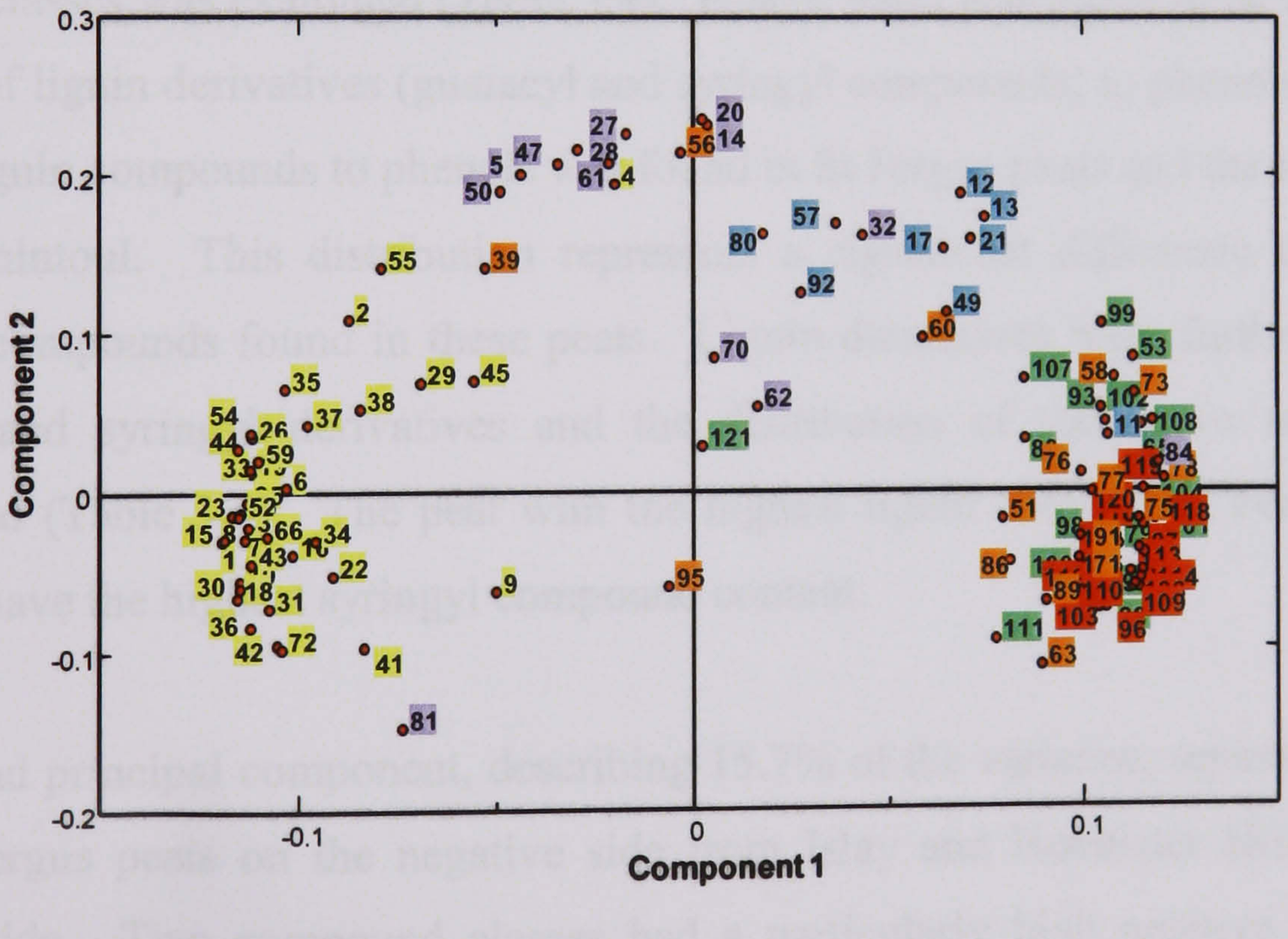


b

Figure 3.7. PCA of Py-GC-MS of peat from different geographical locations. a: Score plot for PCs 1 and 2. Sample codes are described in Chapter 2.1.1. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers and are colour coded according to compound class (Table 2.6).

Table 3.4. Pyrolysis products of peat from different geographical locations.

	Geographical location			
Parameter	**Islay**	**St Fergus**	**Hobbister Hill**	**Tomintoul**
Carbohydrate derivatives (%)	29.79	27.21	47.64	56.85
Guaiacyl compounds (%)	23.86	27.15	15.85	12.75
Syringyl compounds (%)	8.67	16.08	4.51	3.64
Phenols (%)	19.53	18.07	16.27	15.91
Nitrogen-containing compounds (%)	3.41	2.68	2.92	1.28
Aromatic compounds (%)	14.74	8.80	12.81	9.58
Lignin derivatives: phenols [a]	1.68	2.39	1.25	1.03
Syringyl: guaiacyl [b]	0.36	0.59	0.29	0.29
Acetovanillone: *trans*-isoeugenol[c]	2.49	1.23	3.07	1.98

[a] Ratio of total lignin-derived compound peak areas to total non-specific phenols peak area.

[b] Ratio of total syringyl compounds peak area to total guaiacyl compounds peak area.

[c] Ratio of acetovanillone (**99**) peak area to *trans*-isoeugenol (**97**) peak area.

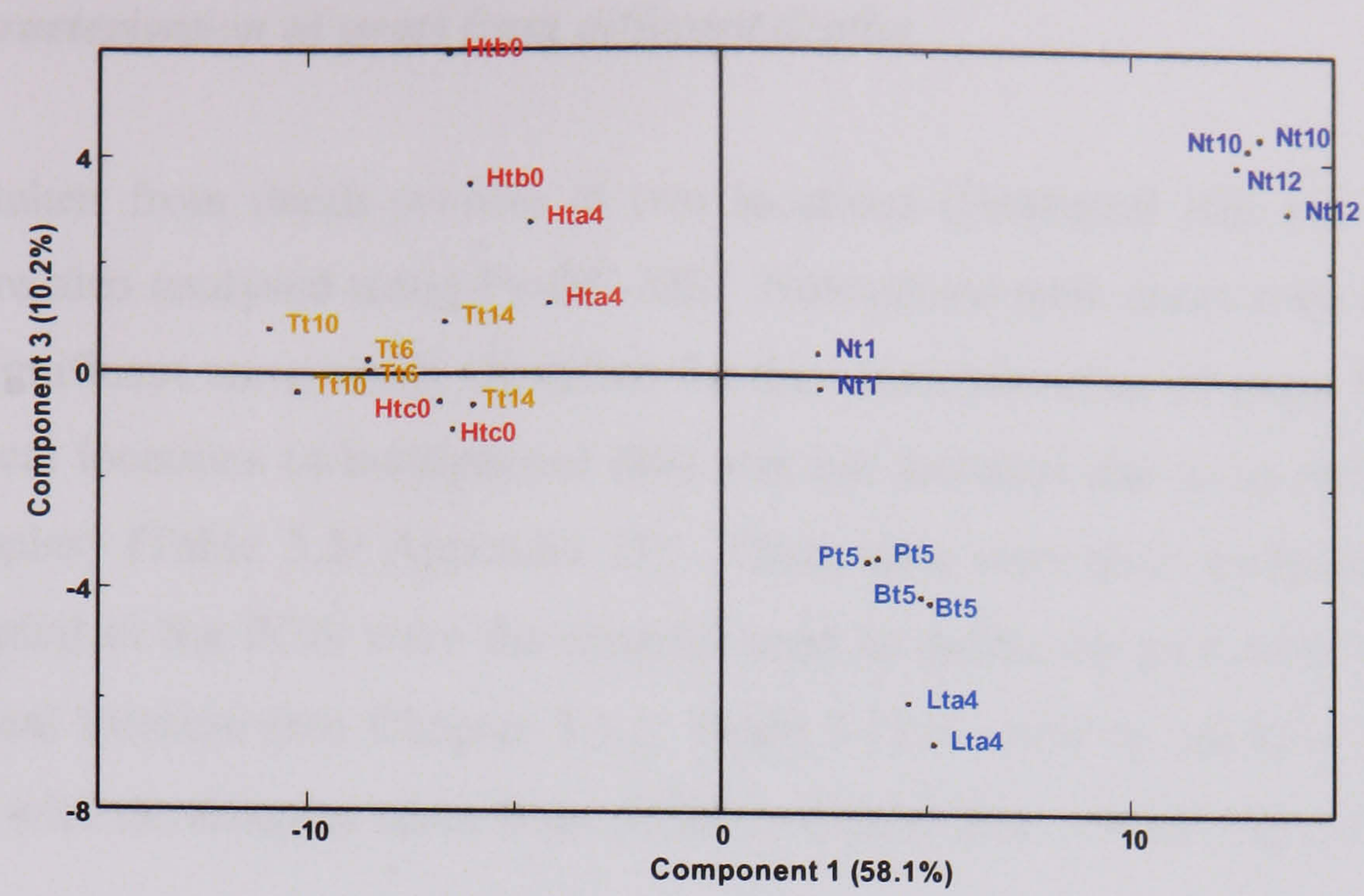
As the phenolic compounds could be divided into several subclasses, the distribution of these subclasses was examined (Table 3.4). Firstly, there was found to be a difference in the ratio of lignin derivatives (guaiacyl and syringyl compounds) to phenols. The highest ratio of lignin compounds to phenols was found in St Fergus peats and the lowest in those from Tomintoul. This distribution represents a significant difference in the type of phenolic compounds found in these peats. Lignin derivatives were further divided into guaiacyl and syringyl derivatives and the distribution of these two subclasses was determined (Table 3.4). The peat with the highest lignin content (St Fergus) was also found to have the highest syringyl compound content.

The second principal component, describing 15.7% of the variance, separated Tomintoul and St Fergus peats on the negative side from Islay and Hobbister Hill peats on the positive side. Two compound classes had a particularly high positive effect on this component. The first class was the nitrogen-containing compounds, with the exception of 1-methyl-1H-pyrrole (**11**) which was relatively abundant in St Fergus peat. The second was a subset of the aromatic compounds class which predominantly consisted of aromatic hydrocarbons (**5**, **14**, **20**, **27**, **28**, **32**, **47**, **50** and **61**). Other aromatic compounds

were widely distributed: 2-methyl-2, 3-dihydrobenzofuran (**81**) was particularly abundant in Tomintoul peat; 1, 3-dimethoxybenzene (**84**) was particularly abundant in St Fergus peat and methoxybenzene (**32**) was particularly abundant in Islay peat. Two aromatic compounds, 4-ethyl-1,2-dimethylbenzene (**62**) and dihydrobenzofuran 2 (**70**) had no strong effects on PCs 1 or 2.

Oxygenated lignin derivatives, notably guaiacyl derivatives, were also found to be higher in Hobbister Hill and Islay peats. The ratio of acetovanillone (**99**) to *trans*-isoeugenol (**97**) has previously been used as a descriptor for the amount of lignin oxidation undergone in peat [47]. This ratio was highest in the Hobbister Hill and Islay samples (Table 3.4).

Fig. 3.8a shows the separation of Islay samples from the rest on PC 3. Explaining 10.2% of the total variance, this was a relatively important separation. The differentiation of the Islay samples was found to be due to a relative abundance of particular compounds in the Islay samples, notably dihydrobenzofuran 2 (**70**), 4-acetylphenol (**95**) and ferulic acid (**121**) (Fig. 3.8b).



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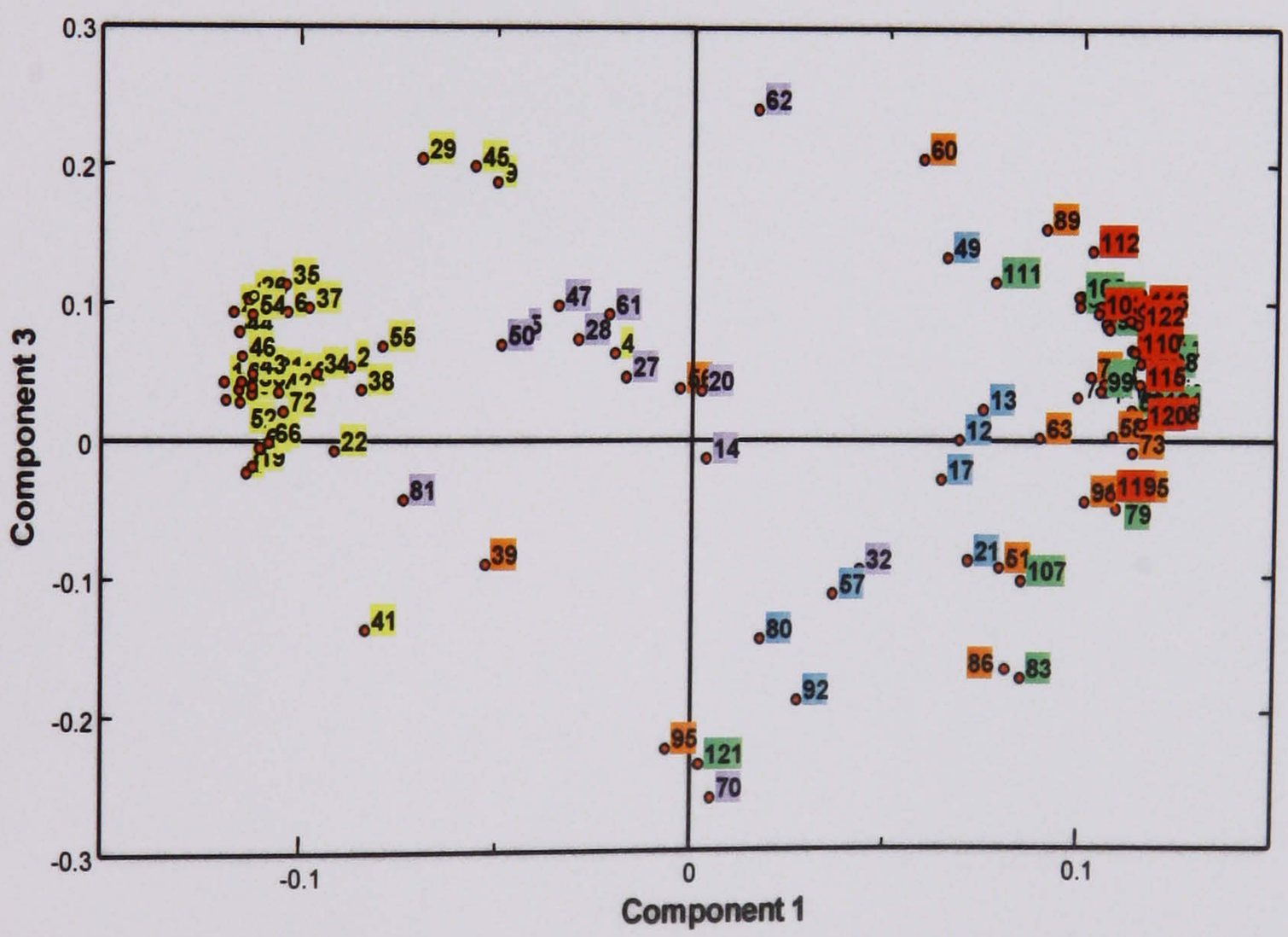
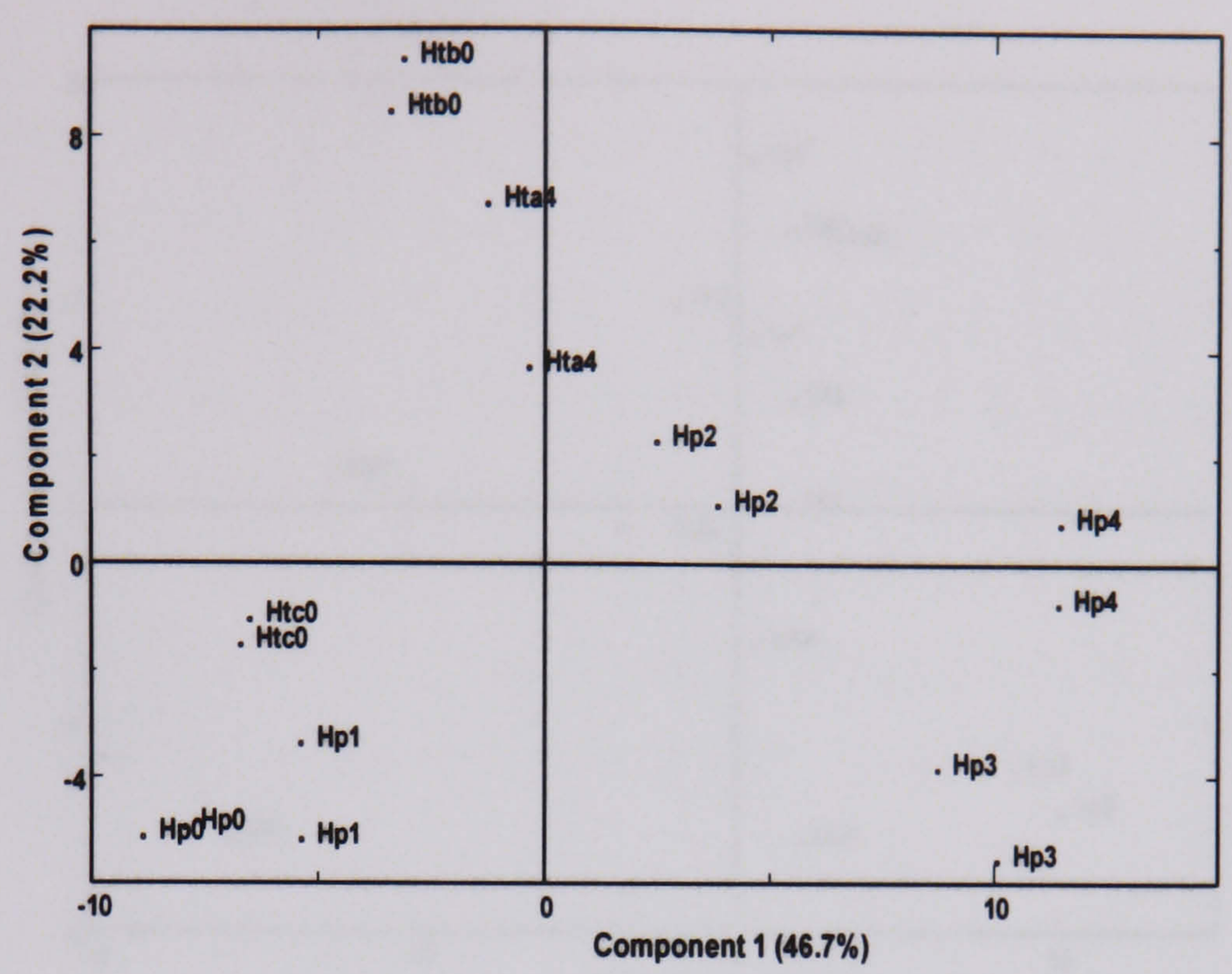


b

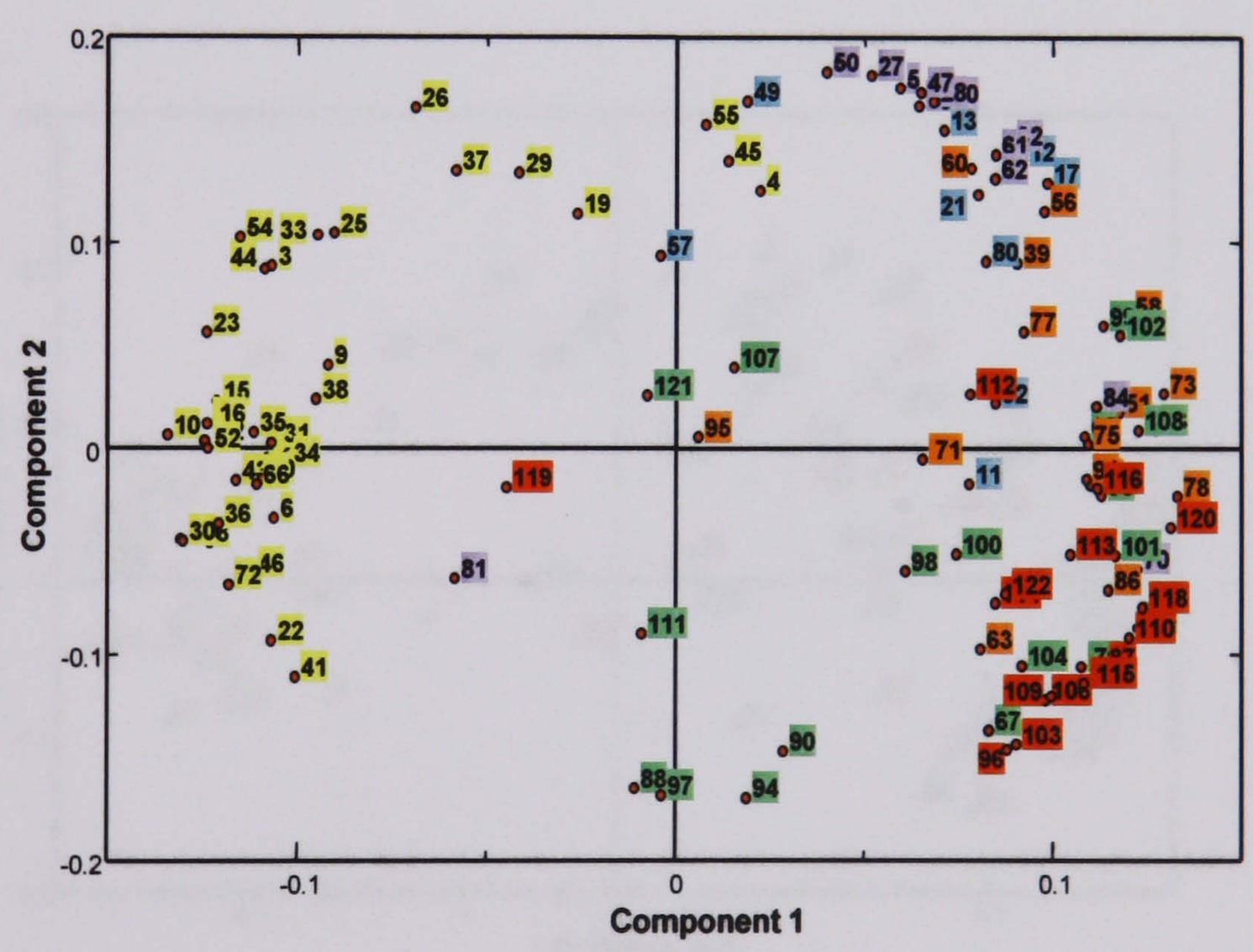
Figure 3.8. PCA of Py-GC-MS of peat from different geographical locations. a: Score plot for PCs 1 and 3. Sample codes are described in Chapter 2.1.1. b: Loadings plot for PCs 1 and 3. Compounds are represented by their peak numbers and are colour coded according to compound class (Table 2.6).

3.1.4 Characterisation of peats from different depths

Samples taken from depth profiles at two locations (Hobbister Hill and Glenmachrie moss) were also analysed using Py-GC-MS. Normalised peak areas were calculated for the 111 significant compounds identified for the characterisation of peats from different geographical locations (4-butylphenol (**89**) was not included due to its poor response in these samples) (Table 3.5/ Appendix D). These data were then analysed using PCA. Also included in the PCA were the samples used to define the peat from the respective geographical location (see Chapter 3.1.2; Table 3.1) in order to see how these samples compared with the samples taken from different depths (Fig. 3.9 and Fig. 3.10).

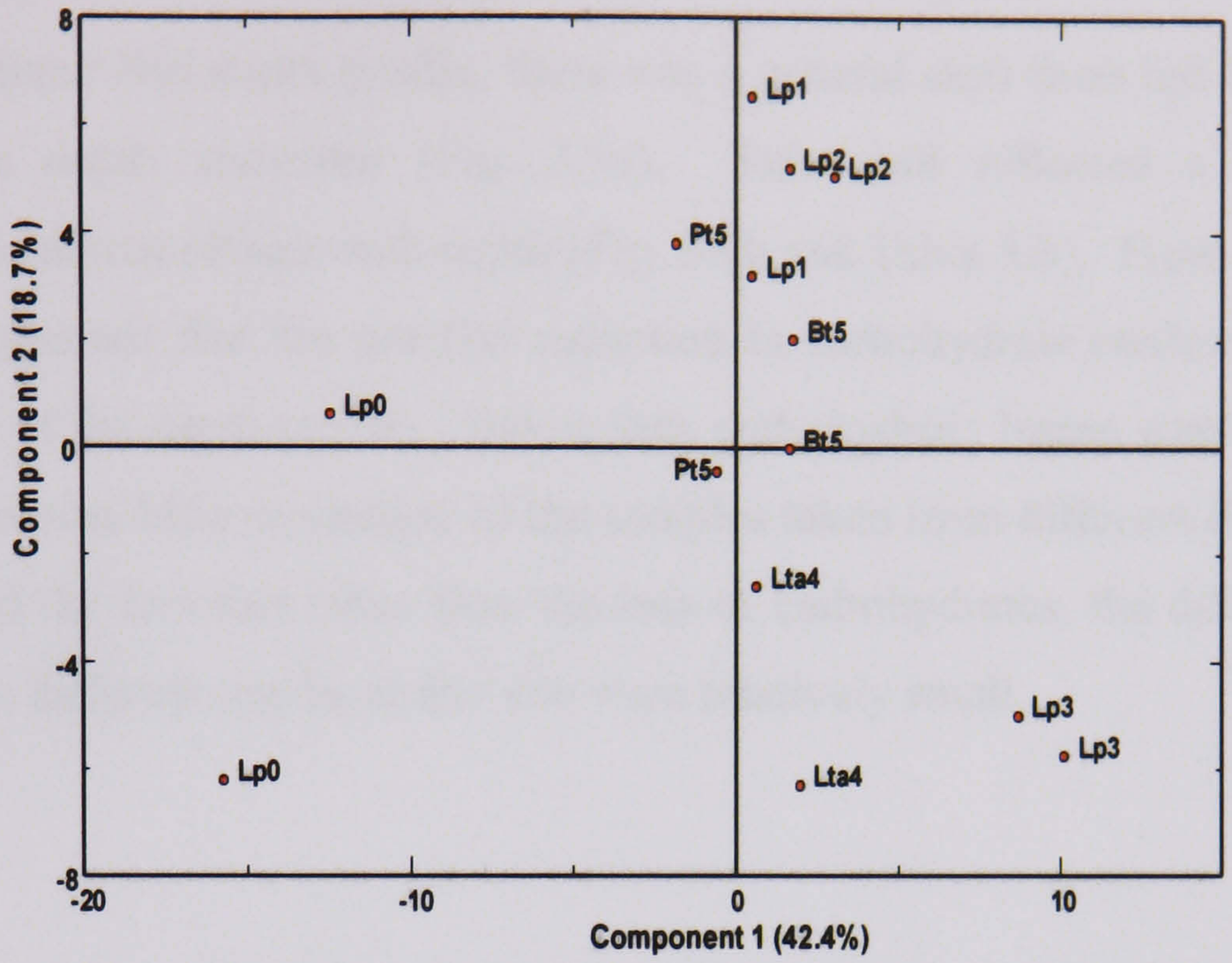


a



b

Figure 3.9. PCA of Py-GC-MS results for Hobbister Hill peat depth profile samples. Also shown are the samples used to characterise Hobbister Hill peat. a: Score plot for PCs 1 and 2. Sample codes are described in Chapter 2.1.1. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers and are colour coded according to compound class (Table 2.6).



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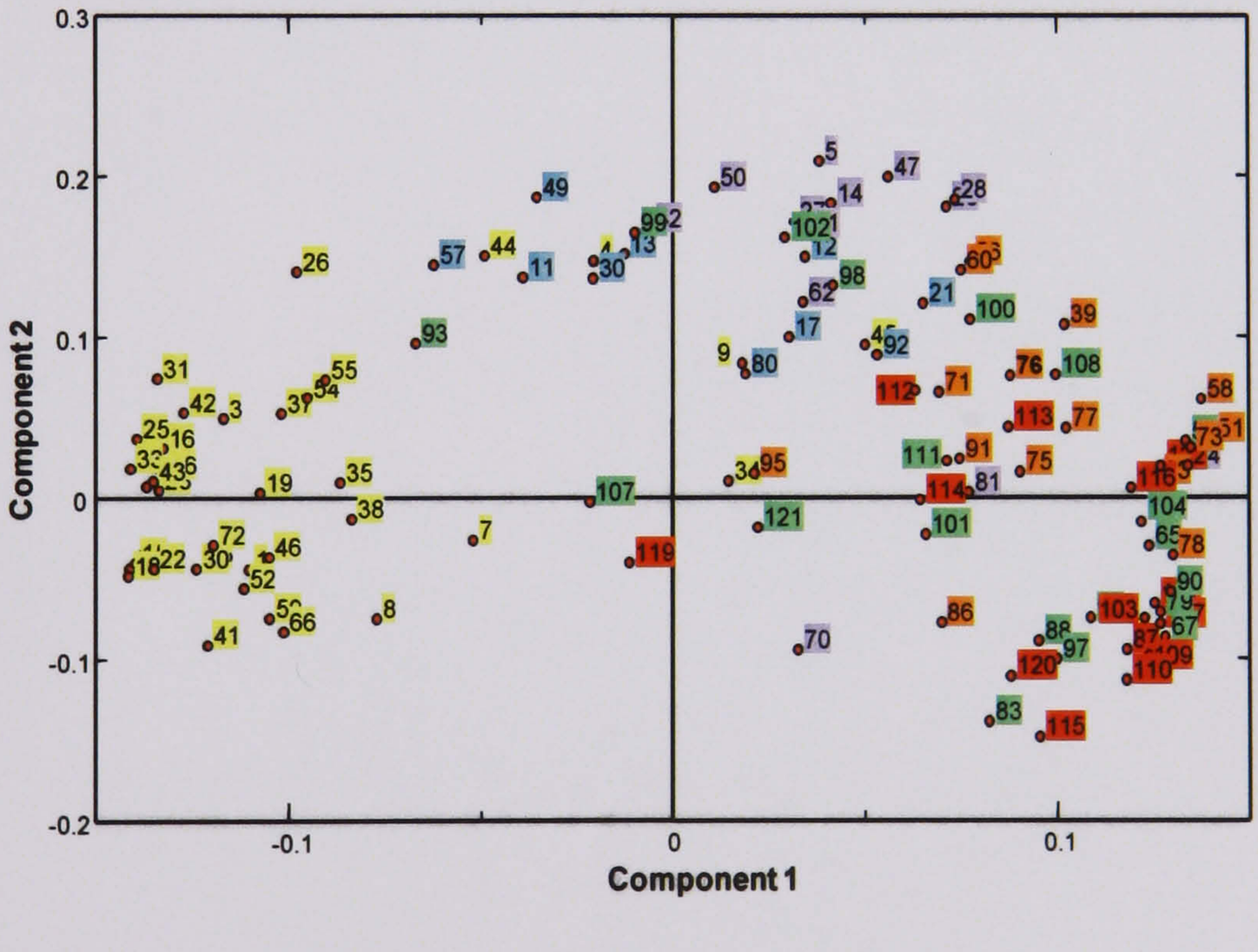


b

Figure 3.10. PCA of Py-GC-MS results for Islay peat samples taken from depth profile. Also shown are the samples used to characterise Islay peat. a: Score plot for PCs 1 and 2. Sample codes are described in Chapter 2.1.1. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers and are colour coded according to compound class (Table 2.6).

Hobbister Hill

For the Hobbister Hill depth profile, there was a general shift from left to right along PC 1 as sample depth increased (Fig. 3.9a). This shift reflected a decrease in the carbohydrate content of peat with depth (Fig. 3.9b and Table 3.6). From the data in Table 3.6, it was apparent that the greatest reduction in carbohydrate content occurred in the upper 60 cm of the depth profile. Below this, carbohydrate losses were relatively small. On PC 2 there was little separation of the samples taken from different depths (Fig. 3.9a). This reflected the fact that other than the loss of carbohydrates, the differences between samples from different depths at this site were relatively small.

Table 3.6. Pyrolysis products of peat from different depths at two sites: Hobbister Hill, Orkney (Hp0 to Hp4) and Glenmachrie moss, Islay (Lp0 to Lp3).

Sample number	Carbohydrate derivatives (%)	Guaiacyl compounds (%)	Syringyl compounds (%)	Phenols (%)	Nitrogen-containing compounds (%)	Aromatics (%)
Hp0 (0 cm)	58.00	17.33	5.43	9.42	1.64	8.17
Hp1 (30 cm)	51.82	16.49	5.11	15.43	1.34	9.81
Hp2 (60 cm)	37.34	18.33	6.19	21.94	3.16	13.04
Hp3 (90 cm)	31.62	24.94	8.35	18.31	2.81	13.97
Hp4 (120 cm)	26.47	23.19	7.39	23.23	3.30	16.42
Lp0 (0 cm)	46.57	19.89	5.75	13.40	2.50	11.89
Lp1 (25 cm)	29.74	23.33	8.06	20.18	3.94	14.74
Lp2 (50 cm)	26.72	23.20	7.75	23.70	3.30	15.33
Lp3 (75 cm)	23.54	26.31	11.40	23.24	2.45	13.07

Sample number	Lignin derivatives: phenols	Syringyl: guaiacyl	Acetovanillone: *trans*-isoeugenol
Hp0 (0 cm)	2.42	0.31	1.31
Hp1 (30 cm)	1.41	0.31	2.16
Hp2 (60 cm)	1.12	0.34	2.23
Hp3 (90 cm)	1.82	0.33	2.22
Hp4 (120 cm)	1.32	0.32	3.50
Lp0 (0 cm)	1.96	0.29	2.45
Lp1 (25 cm)	1.56	0.35	2.67
Lp2 (50 cm)	1.31	0.33	2.54
Lp3 (75 cm)	1.62	0.43	1.39

Islay

The distribution of samples in the Islay profile was similar to that of the Hobbister Hill profile in that, with increasing depth, samples moved from negative to positive along PC 1 (Fig. 3.10a). As with the Hobbister Hill depth profile, this shift reflected a decrease in the proportion of carbohydrate compounds with increasing depth (Fig. 3.10b and Table 3.6). Here again, the carbohydrate loss was greatest in the upper horizon of the peat profile, in this case the upper 25 cm. Along PC2, Lp0 and Lp3 (the shallowest and the deepest samples respectively) were located on the negative side whereas the middle two samples, Lp1 and Lp2, were located on the positive side (Fig. 3.10a). This distribution reflected a higher proportion of nitrogen-containing compounds and most aromatic compounds (dihydrobenzofuran 2 (**70**), 2-methyl-2,3-dihydrobenzofuran (**81**), and 1,3-dimethoxybenzene (**84**) did not follow this trend) in Lp1 and Lp2 compared with Lp0 and Lp3 (Fig. 3.10b).

3.1.5 Summary

Using FT-IR, it was possible to distinguish between peats from different geographical locations. From the pattern of the thermal degradation products analysed by Py-GC-MS, it was possible to deduce the chemical structures responsible for this differentiation. Peats from Hobbister Hill and Tomintoul were found to be rich in carbohydrate derivatives while peats from Islay and St Fergus contained higher levels of lignin-derived compounds. In addition, peats from Hobbister Hill and Islay were richer in aromatic hydrocarbons and nitrogen-containing compounds respectively.

3.2 Chemical Profiles of Peated Malts

3.2.1 Introduction

Having established the chemical differences between peats from different geographical locations, it was necessary to determine if these differences were evident in peated malt produced using these peats. Peated malt was therefore produced on a laboratory scale using composites of the transect samples collected from the six sampled geographical locations. The peated malts were initially analysed for their marker phenols content to determine whether these commonly used peating level indicators could be used to differentiate peats from different locations. The peated malts were subsequently analysed more broadly for all compounds belonging to the classes identified in the peat pyrolysis work, and the effect of peat source on peated malt composition was thus determined. Additionally, industrially produced peated malts were analysed to ascertain the impact of industrial processing variations on the differentiation due to peat source.

3.2.2 Marker phenols profiles of lab-scale peated malts

Head-Space Solid-Phase-Micro-Extraction in conjunction with GC-MS (HS-SPME-GC-MS) was used to analyse the eight quantitatively important marker phenols in the lab-scale peated malt produced using peat from the six sampled peat locations. A typical chromatogram is shown in Fig. 3.11.

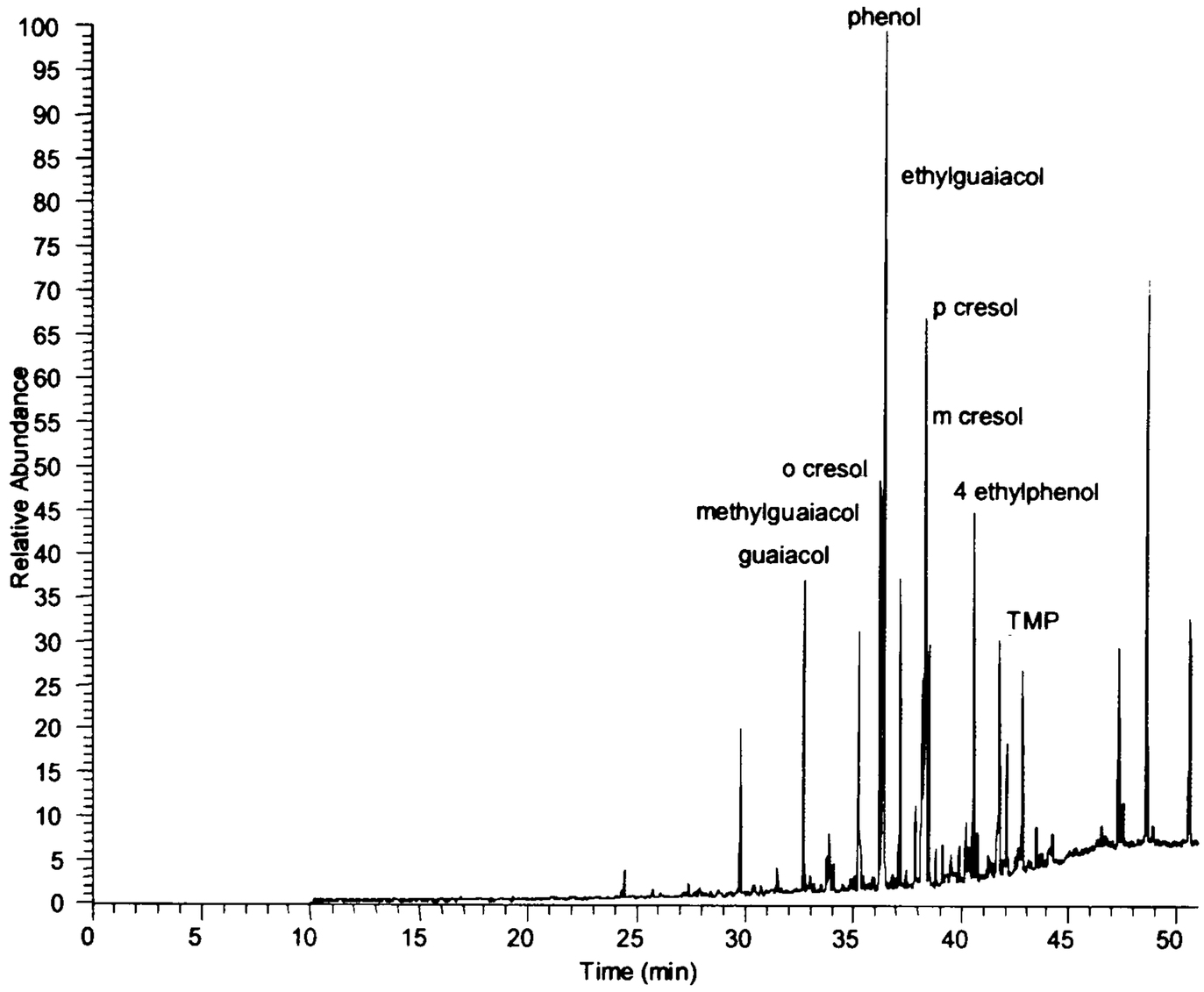
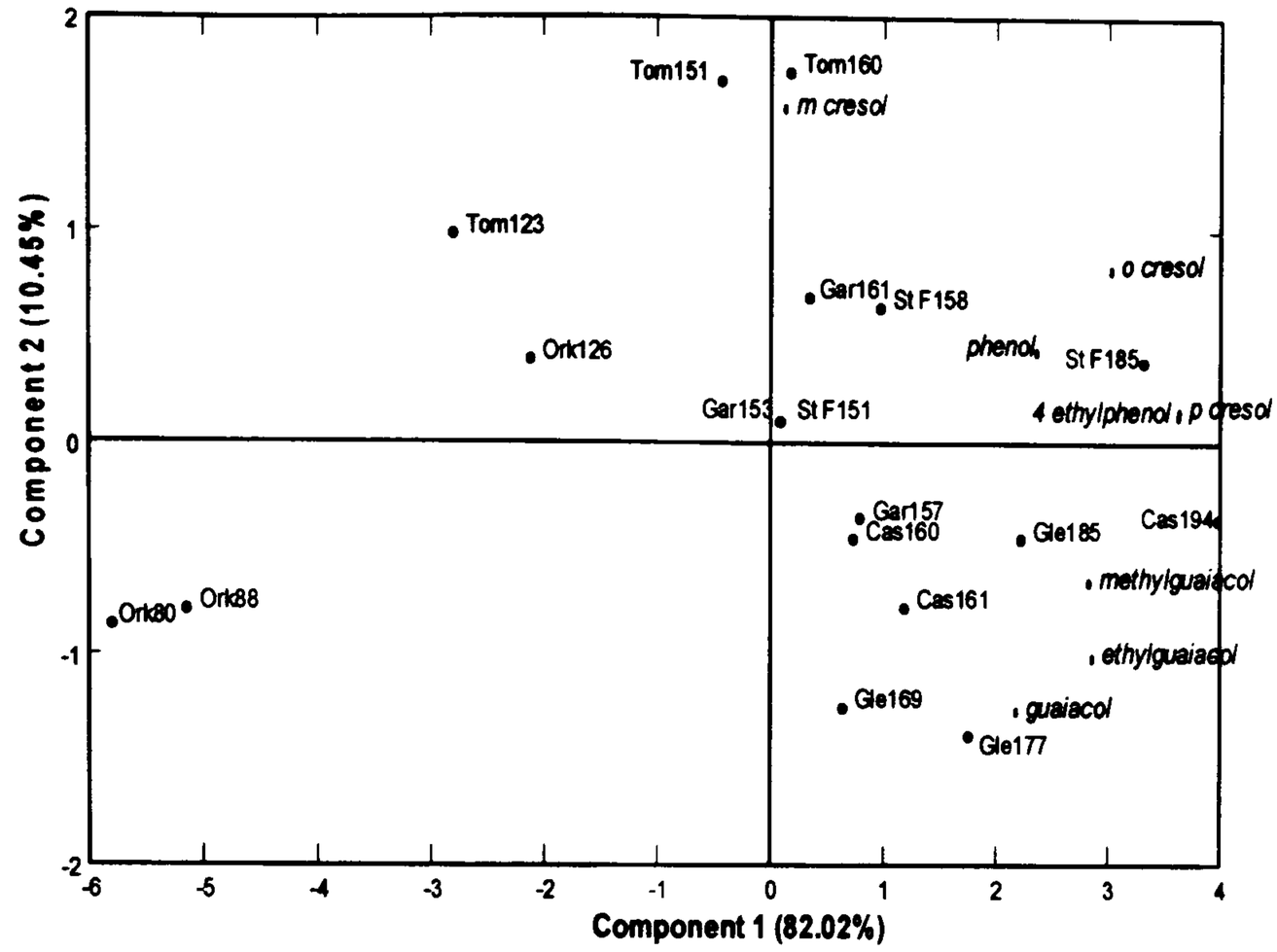


Figure 3.11. Chromatogram produced from HS-SPME-GC-MS analysis of Glenmachrie peated malt. M/z shown = 93.50–94.50, 106.50–107.50, 107.50–108.50, 108.50–109.50, 120.50–121.50, 121.50–122.50, 122.50–123.50, 123.50–124.50, 135.50–136.50, 136.50–137.50, 137.50–138.50 and 151.50–152.50.

Concentrations of the marker phenols in the lab-scale peated malt samples are shown in Table 3.7. These data were summarised using PCA and the resulting contribution of these phenols to the discrimination of the malt samples is displayed in Fig. 3.12.

Table 3.7. Concentrations of marker phenols in peated malt samples. Number of samples per malt = 3. Concentrations are given in mg/kg of malt (values in brackets are % RSD).

Compound	Castlehill (Cas)	Garbreck (Gar)	Glenmachrie (Gle)	Hobbister (Ork)	St Fergus (St F)	Tomintoul (Tom)
Guaiacol	19.3 (9.5)	16.4 (7.1)	19.1 (5.3)	9.6 (20.5)	17.1 (13.1)	9.7 (13.5)
Methylguaiacol	16.2 (12.4)	13.0 (7.3)	16.2 (5.3)	8.7 (18.2)	17.4 (14.5)	10.4 (14.0)
***o*-Cresol**	8.8 (9.4)	8.7 (2.5)	8.5 (6.5)	6.0 (26.5)	8.8 (11.8)	8.3 (12.1)
Phenol	69.2 (9.8)	67.8 (6.2)	78.3 (4.1)	41.6 (29.1)	65.6 (10.0)	68.2 (13.0)
Ethylguaiacol	8.3 (14.2)	6.6 (11.3)	7.9 (7.2)	4.4 (14.6)	7.3 (14.1)	5.1 (13.0)
***p*-Cresol**	27.8 (14.0)	24.7 (2.9)	27.9 (9.3)	14.4 (24.9)	25.5 (11.3)	23.3 (14.9)
***m*-Cresol**	10.2 (12.0)	9.6 (5.0)	8.1 (9.8)	6.6 (28.4)	12.2 (9.9)	10.2 (12.7)
4-Ethylphenol	11.8 (14.7)	10.4 (3.1)	10.8 (10.1)	6.6 (19.4)	10.6 (10.6)	9.5 (14.1)
Total phenols	171.7 (11.1)	157.2 (2.3)	176.8 (4.5)	97.8 (25.1)	164.6 (11.2)	144.7 (13.3)



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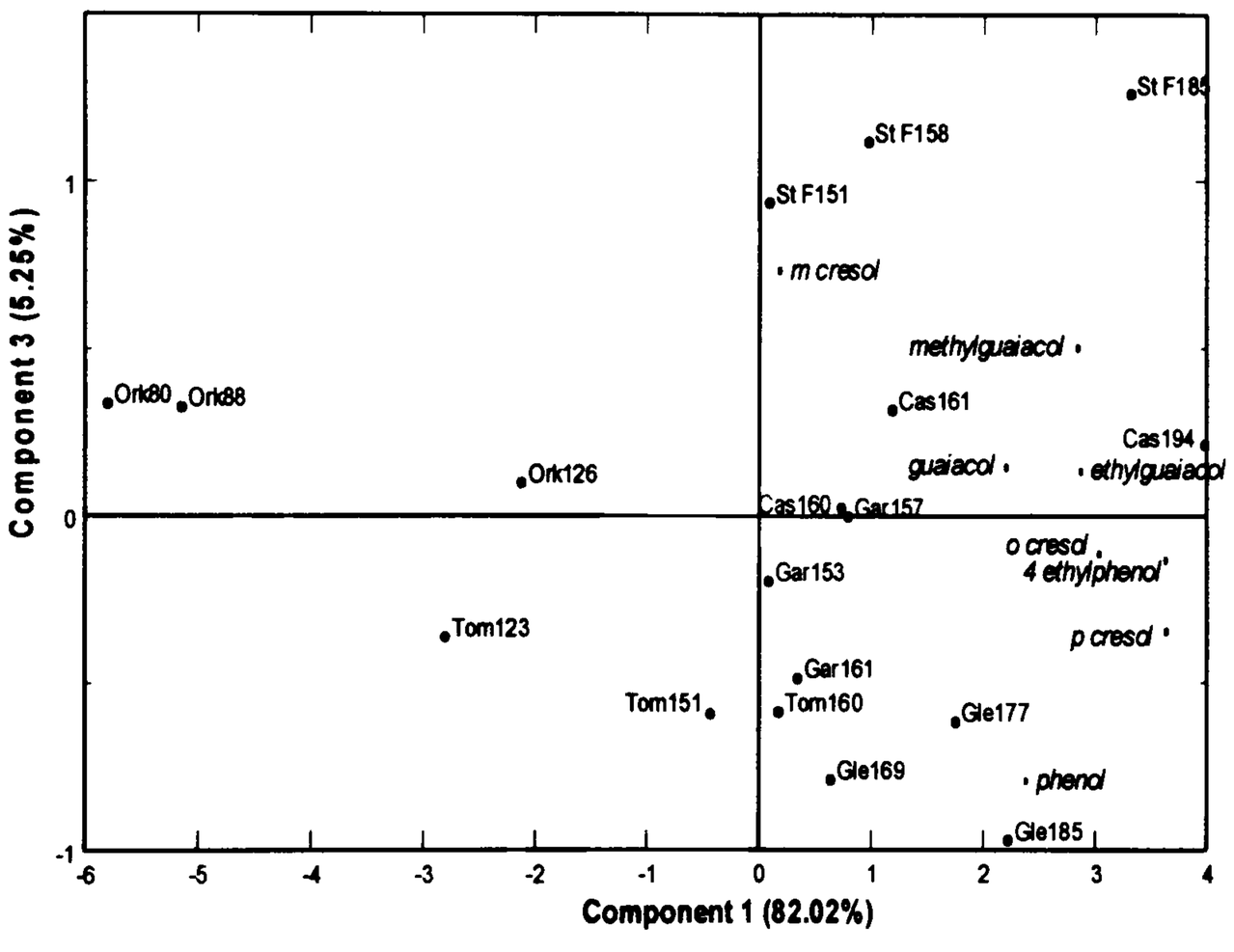


b

Figure 3.12. PCA of marker phenol concentrations in lab-scale peated malt. Sample codes are as described in Table 3.7 and numbers after sample codes refer to total marker phenols levels. a: Bi plot of PCA scores and loadings for PCs 1 and 2. b: Bi plot of PCA scores and loadings for PCs 1 and 3.

The main source of variation amongst the peated malt samples, as represented by PC 1, was the overall level of marker phenols (Fig. 3.12a). Differences were detected depending on the geographical source of the peat used, despite there being a degree of variation within each source. Tomintoul and Hobbister Hill peat gave malts with relatively low overall phenol levels.

PC 2 represented a difference in the ratio of the guaiacols to the other compounds and in particular to *m*-cresol (Fig. 3.12a). Malts produced using Glenmachrie and Castlehill peats were characterised by relatively high levels of guaiacols, whilst Tomintoul peat gave relatively high levels of *m*-cresol.

The differentiation represented by PC 3 also concerned the relative abundance of *m*-cresol (Fig. 3.12b). In this case however, it was the abundance of *m*-cresol and the guaiacols, most notably methylguaiacol, relative to the other compounds which caused the separation. In this regard, St Fergus peat produced a malt with a relatively high abundance of *m*-cresol and the guaiacols.

3.2.3 Marker phenols profiles of industrial peated malts and comparison with lab-scale samples

The concentrations of marker phenols in a range of industrial peated malts, produced using the same peat sources as the lab-scale samples, are given in Table 3.8.

Table 3.8. Concentrations of marker phenols in a range of industrial peated malt samples. Concentrations are given in mg kg^{-1} of malt. For sample names, letters are used where more than one sample was obtained from the same malting (For Orkney and Tomintoul replicates were taken from the same sample).

Sample	Guaiacol	Methylguaiacol	*o*-Cresol	Phenol	Ethylguaiacol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	Total phenols
Cas (Castlehill) a	2.6	2.0	3.9	25.7	0.9	12.7	4.9	5.2	58.0
Cas b	2.1	1.7	3.8	27.9	0.6	11.9	4.4	4.3	57.0
Cas c	4.1	3.0	6.6	49.0	1.6	21.9	8.7	8.6	103.0
Cas d	2.6	2.1	4.5	31.8	0.9	13.9	5.6	5.5	67.0
Cas e	2.9	2.4	4.8	34.0	1.1	15.6	6.2	6.2	73.0
Cas f	3.1	2.3	5.3	39.6	1.1	17.9	7.0	6.7	83.0
Gar (Gartbreck) a	0.2	0.8	0.5	2.5	0.0	1.1	0.6	0.5	6.0
Gar b	0.2	0.8	0.5	2.8	0.0	1.2	0.6	0.5	6.0
Gle (Glenmachrie) a	2.3	1.9	5.1	46.0	0.8	16.0	6.3	5.9	84.0
Gle b	2.3	1.8	5.0	43.6	0.7	15.5	6.2	5.9	81.0
Ork (Hobbister hill) a	0.5	1.0	1.3	9.4	0.2	3.8	1.8	1.3	19.0
Ork b	0.5	1.0	1.3	9.7	0.1	3.9	1.8	1.3	19.0
St F (St Fergus) a	2.1	2.1	4.0	31.5	0.8	10.4	5.8	3.8	60.0
St F b	1.9	2.0	3.2	22.8	0.6	8.9	4.8	3.3	47.0
Tom (Tomintoul) a	2.3	2.1	8.6	56.8	0.9	18.4	9.3	6.9	105.0
Tom b	2.6	2.3	9.1	62.0	1.0	19.6	10.0	7.3	114.0

Comparing the data in Table 3.8 with those presented in Table 3.7, it was found that the industrial peated malts were generally peated to lower levels than the lab-scale equivalents. Also, when the marker phenols concentrations were normalised as a percentage of the total concentration, a difference in the proportions of marker phenols present could be seen between industrial and lab-scale samples (Fig. 3.13). Clearly, there were higher proportions of guaiacols in the lab-scale samples than in the industrial samples.

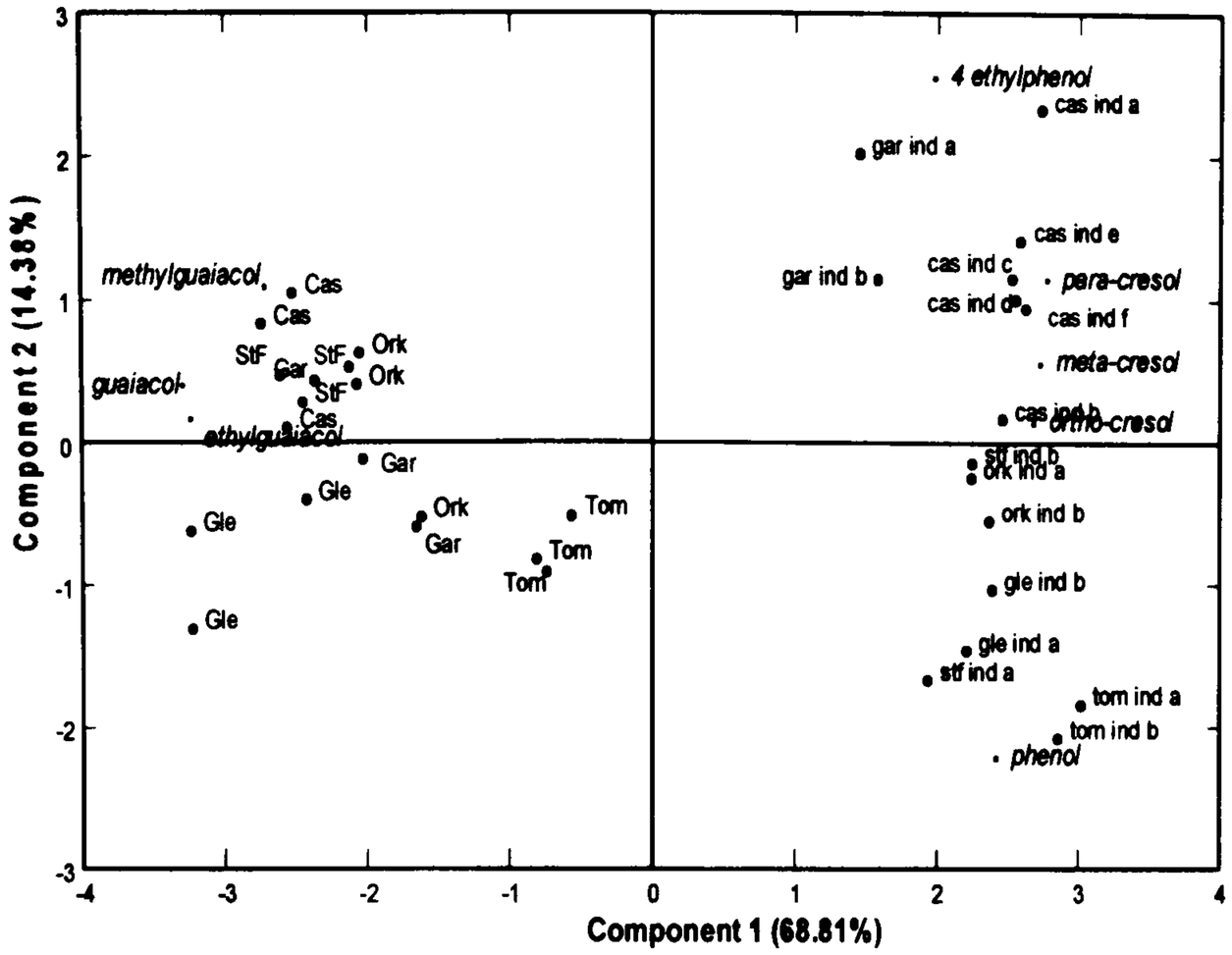


Figure 3.13. PCA bi plot of normalised marker phenols concentrations in industrial and lab-scale peated malt samples. Sample codes are defined in Tables 3.7 and 3.8.

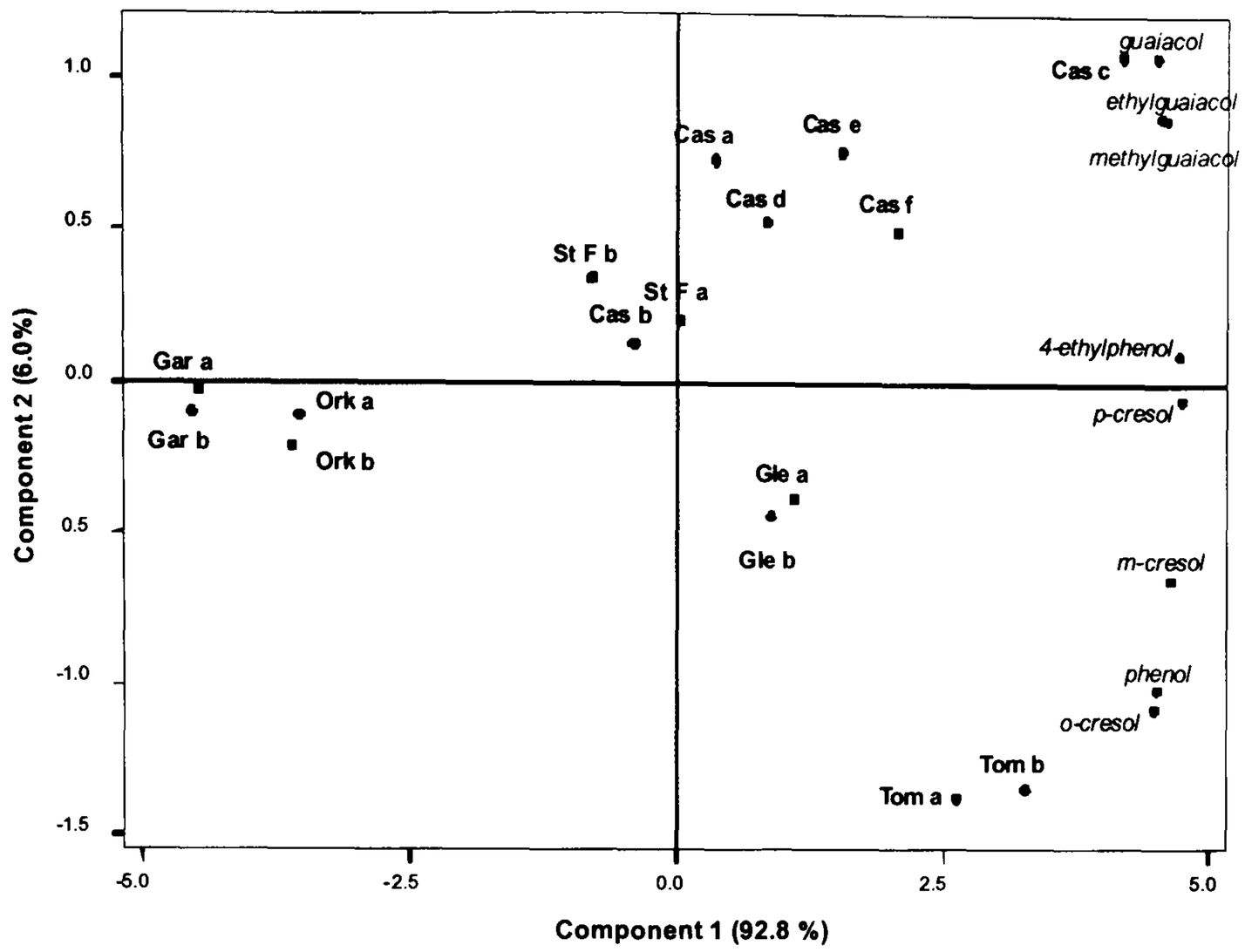
Given the distinction in the composition of the lab-scale and industrial samples, PCA was used to compare the levels of the marker phenols in the industrial samples alone (Fig. 3.14). The resultant principal components were then analysed for patterns similar to those found in the lab-scale data set.

The separation on PC 1 reflects the fact that the major influence on the composition of the industrial samples was the overall peating level (Fig. 3.14a). This accounted for the vast majority of the variance among samples (92.8%). Whilst a similar finding was made in the lab-scale data set, the separation of samples in the two data sets could not be correlated given the unknown variation in the volumes of peat used for industrial peating.

Though they only explained very small relative amounts of variance, subsequent components did show some similar separations to those found in the lab-scale data set. PC 2 separated the Tomintoul peated malt from the rest (Fig. 3.14a). As was the case with the lab-scale samples, the industrial Tomintoul samples contained a relatively low abundance of guaiacols and a relatively high abundance of *m*- and *o*-cresol as well as

phenol. The distribution of the rest of the samples on this component was not as well correlated between the lab-scale and industrial samples.

PC 3 also showed a similar pattern to that found in the lab-scale samples (Fig. 3.14b). In both data sets, St Fergus peated malt was distinguished by a relatively high abundance of *m*-cresol and methylguaiacol whilst Glenmachrie peated malt was characterised by a relatively low abundance of these compounds. Here again though, the remaining separations were less well correlated between the lab-scale and industrial samples.



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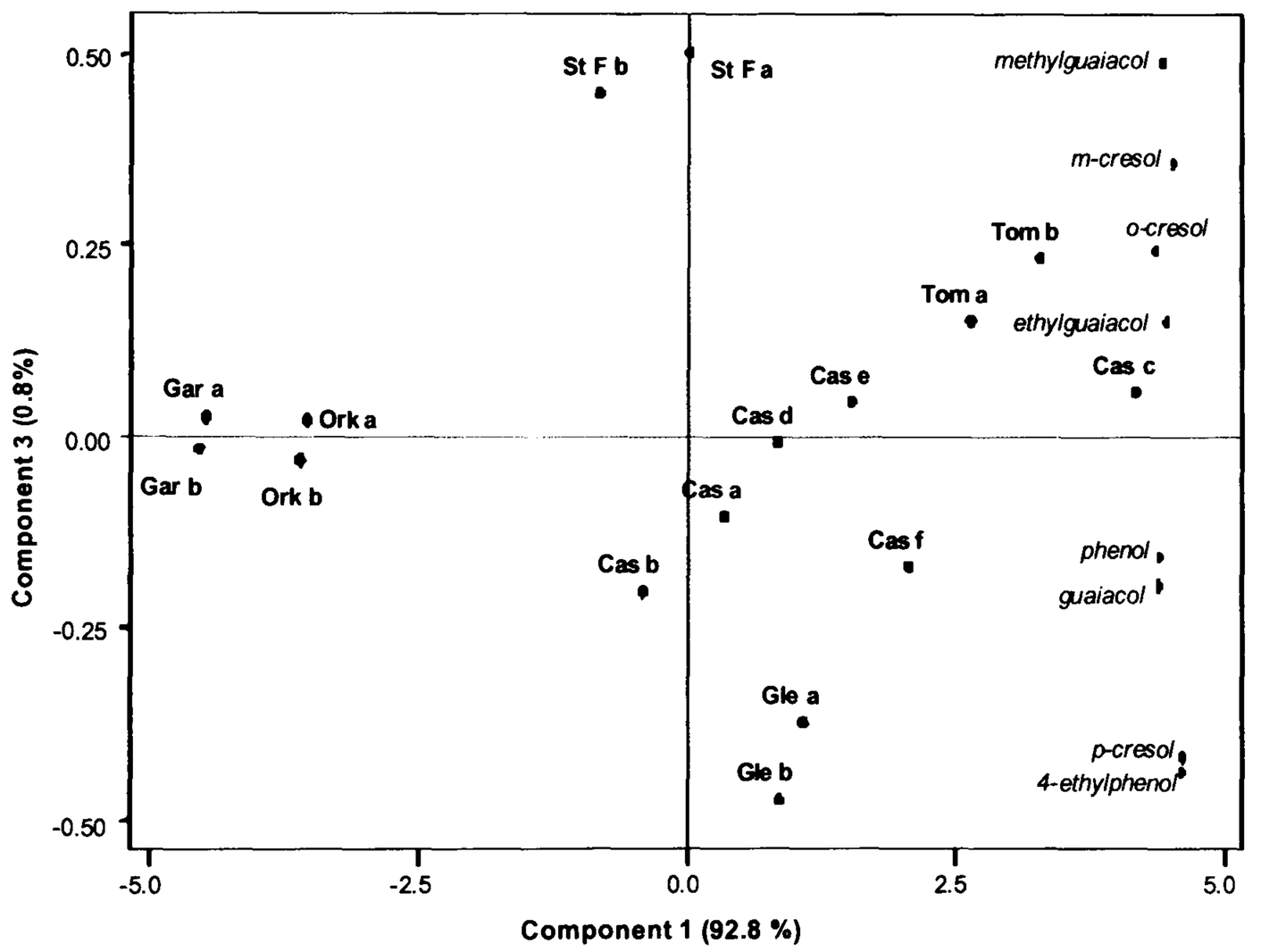


b

Figure 3.14. PCA of marker phenol concentrations in industrial peated malt. Sample codes are defined in Table 3.8. a: Bi plot of PCA scores and loadings for PCs 1 and 2. b: Bi plot of PCA scores and loadings for PCs 1 and 3.

3.2.4 Total chemical profiles of lab-scale malts

The same peated malt extracts that were analysed by HS-SPME-GC-MS in Chapter 3.2.1 were subjected to SPE-GC-MS. Using SPE-GC-MS, 138 peat-derived compounds were identified in lab-scale peated malts in addition to the eight marker phenols. The compound classes analysed in peated malt were the same as those identified in peat using Py-GC-MS in Chapter 3.1.2, namely: lignin markers (guaiacols and syringols), phenols, carbohydrate derivatives, aromatic compounds and nitrogen-containing compounds. The particular compound species detected were not the same in peated malt as they were in peat. The identities of the 138 peat-derived compounds identified using SPE-GC-MS can be found in Table 3.9 (Appendix E) along with the marker phenol data obtained using HS-SPME-GC-MS. The estimated retention indices listed in Table 3.9 were calculated as described in Chapter 2.4.2 and Fig. 3.15 shows the plot of literature retention index data for compounds identified here with a high degree of certainty by MS versus experimental retention time data.

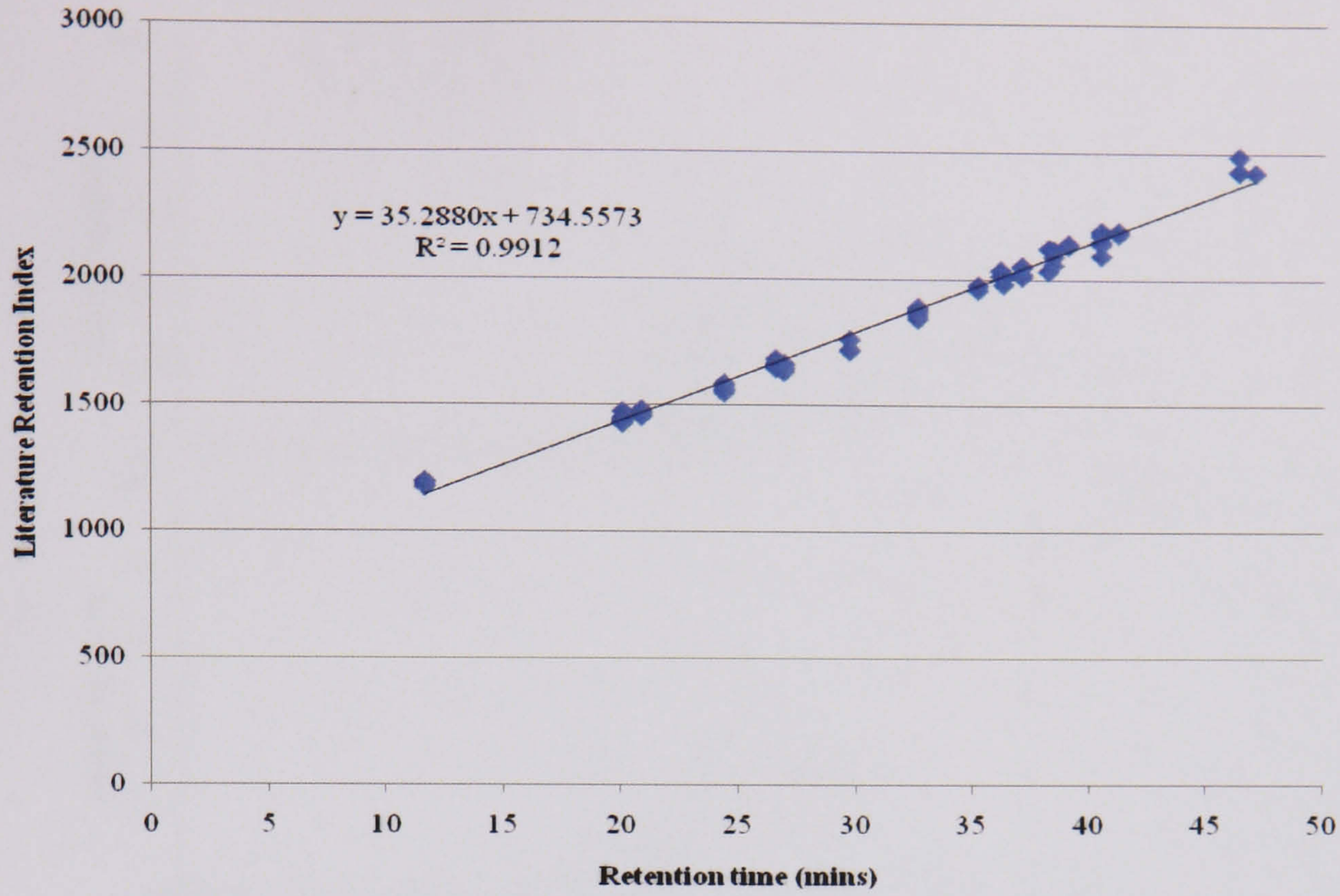
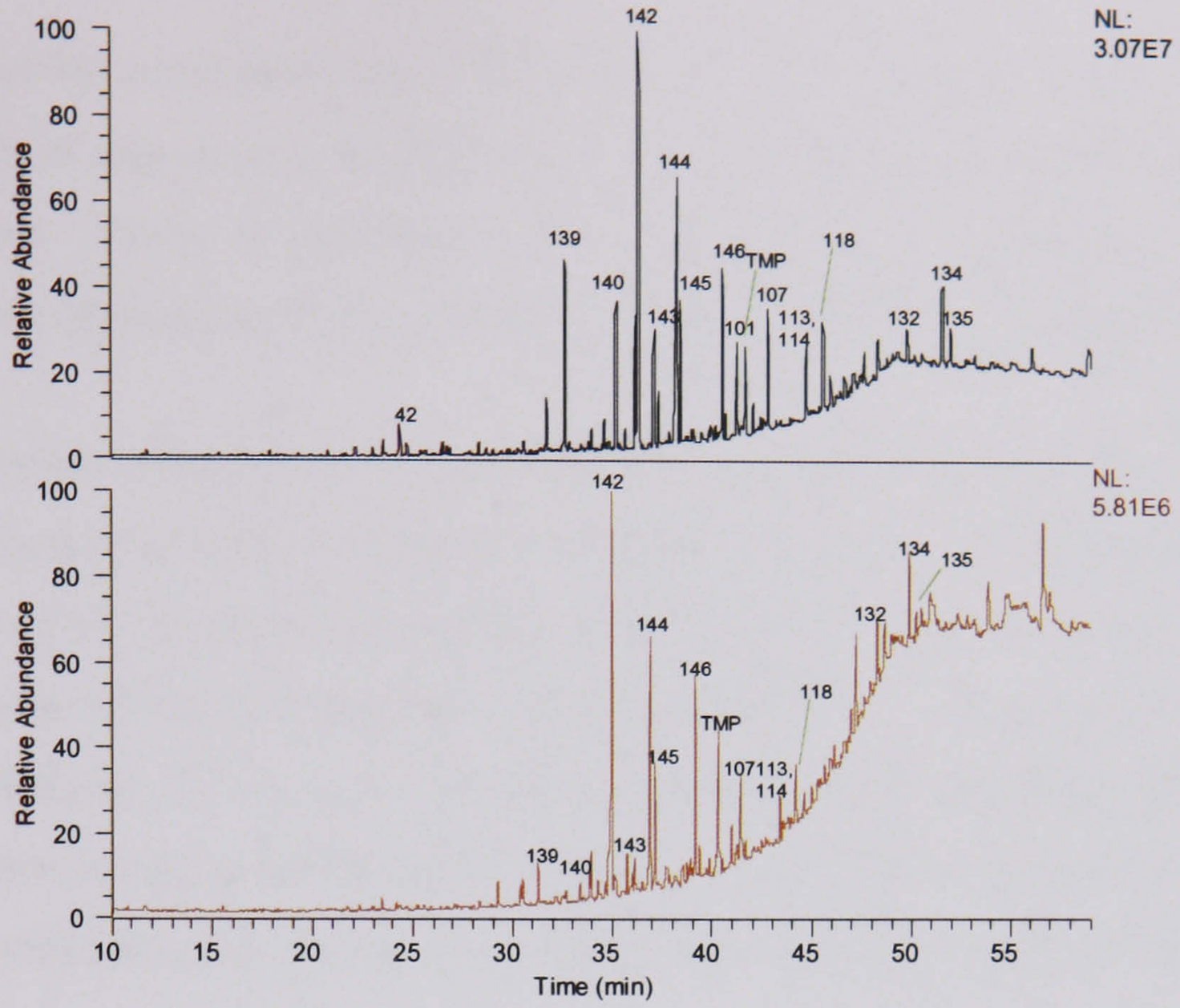


Figure 3.15. Plot of literature retention index data for compounds identified in malt solid phase extracts with a high degree of certainty by MS versus experimental retention time data.

The levels of the peat-derived compounds measured in the lab-scale samples are shown in Table 3.10 (Appendix F). A chromatogram obtained from a lab-scale sample analysed using SPE-GC-MS is compared with that from a heavily peated industrial sample made using peat from the same source in Fig. 3.16.

The compounds detected in peated malt were all confirmed as being derived from peat by using ANOVA to compare the levels recorded in peated malts with those found in an unpeated lab-scale malt sample. Some of the compounds were detected in unpeated malt but at significantly lower levels than in peated malt. Low levels of naphthalenes (**57**, **67** and **70**) and phenol (**142**) appeared to be artefacts of the extraction method as they were also detected in blank samples which had not been in contact with malt.



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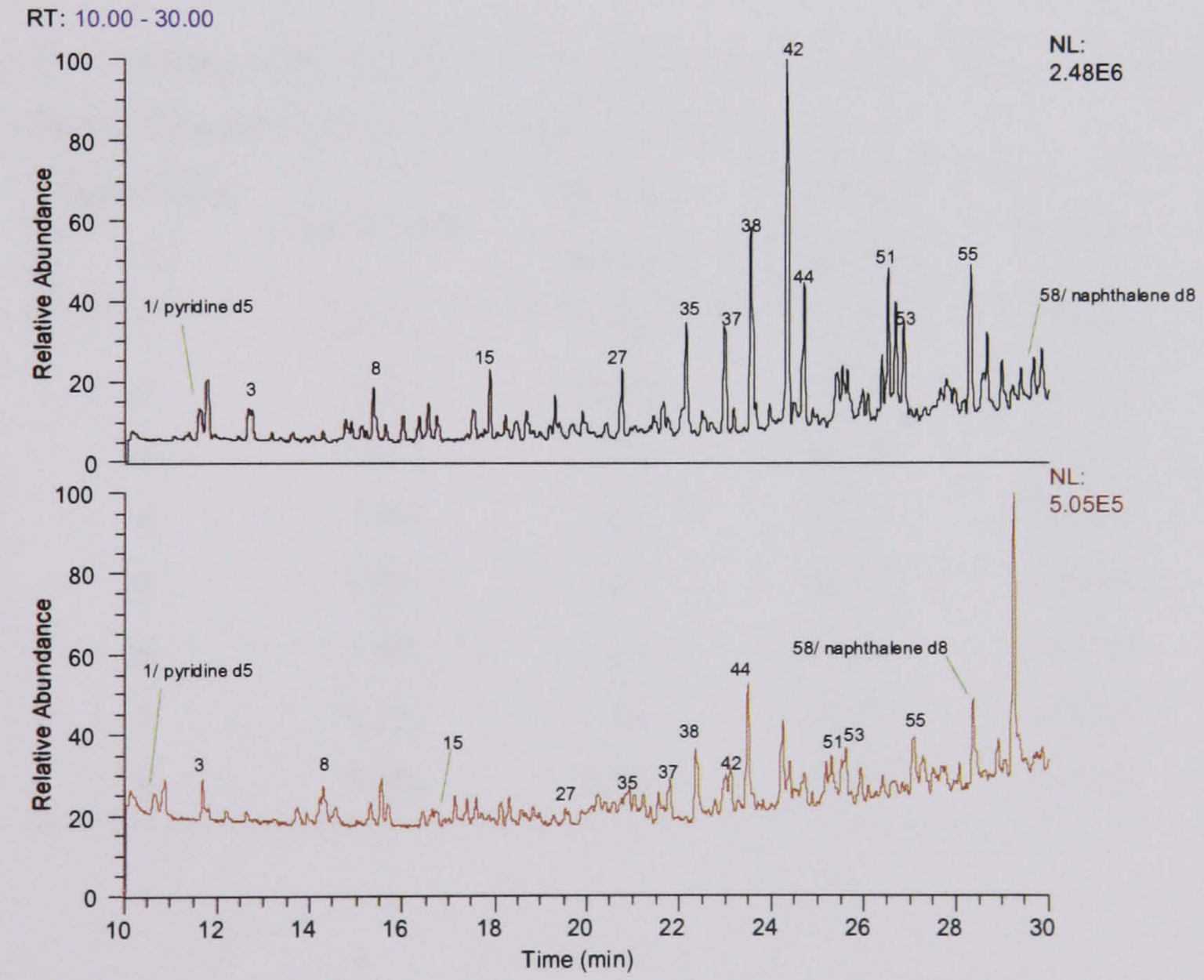

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Figure 3.16. Chromatograms obtained from SPE-GC-MS of lab-scale and industrial peated malt samples produced using Castlehill peat (black= lab-scale, red= industrial sample). a: total ion chromatogram, b: early eluting components.

To assess which compounds were important for characterising the six lab-scale peated malts, ANOVA was used to detect those compounds which were significantly different in concentration. Those 38 compounds which did not show a significant difference were removed from subsequent analysis and are identified in Table 3.10 (Appendix F).

The data for the 108 significant compounds were analysed using PCA. This analysis produced seven principal components with eigen values of 1 or higher (Table 3.11). To determine which of these components were important for describing the difference between the six different peated malt samples, ANOVA was used using peat source as a factor. In this way, PCs 1, 2, 3, 4 and 6 were found to show significant differentiation of samples produced using different peat (Table 3.11). Components 4 and 6, however, only explained relatively low levels of variance and so were excluded from subsequent analysis.

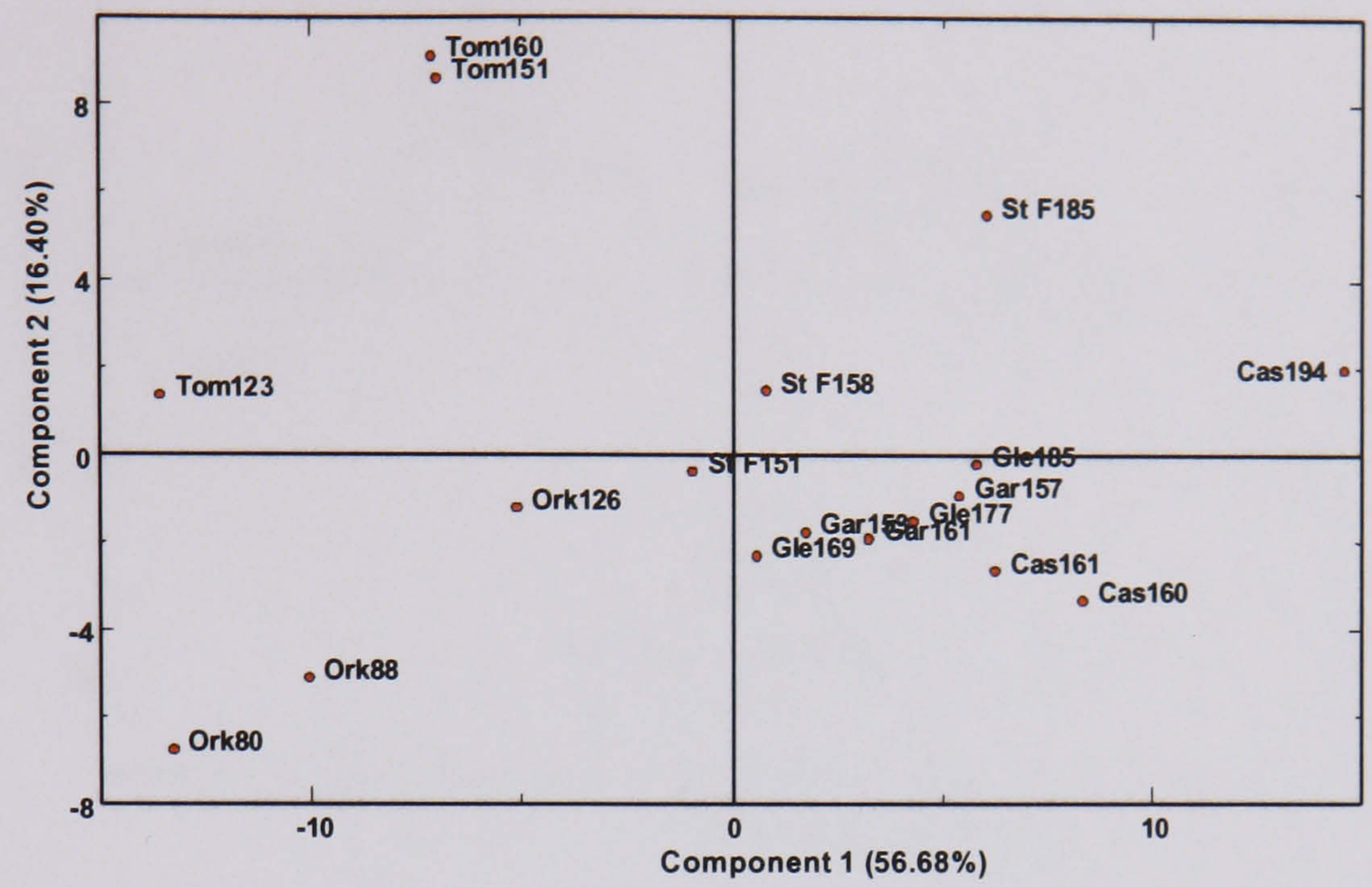
Table 3.11. Variance table for PCA of peat-derived compounds in malt. P values refer to results of ANOVA carried out on PCs using peat source as a factor.

Component No.	Eigen value	Percent variance	Cumulative % variance	p value
1	61.21	56.68	56.68	0.0001
2	17.72	16.40	73.08	0.0072
3	15.52	14.37	87.45	0.0000
4	3.99	3.69	91.14	0.0003
5	2.39	2.21	93.36	0.8573
6	1.89	1.75	95.11	0.0473
7	1.36	1.26	96.37	0.8678
8	1.06	0.98	97.35	0.5792

PCs 1 and 2 are plotted in Fig. 3.17. On PC 1, Hobbister Hill and Tomintoul samples had very similar negative values. On the other hand, St Fergus and Islay samples had positive values. Compounds which had positive loadings on PC 1, which were therefore characteristic of St Fergus and Islay samples, were the nitrogen-compounds, the lignin

derivatives and most of the phenols. Only two phenols had a negative value for PC 1, 2-chlorophenol (**66**) and C3 phenol 6 (**103**). Compounds with negative loadings for PC 1, which were therefore characteristic of Tomintoul and Hobbister Hill samples, were predominantly carbohydrate-derived compounds. Two carbohydrate compounds were exceptions to this finding, being located on the positive side of PC 1: 2-methylcyclopentanone (**2**) and 3-hydroxy-2,6-dimethyl-4H-pyran-4-one (**85**). A subset of aromatic compounds was also found to have negative values on PC 1: benzofuran (**34**), methylindanone 3 (**95**), methylindanone 4 (**100**), dibenzofuran (**109**), and 2-methyl-2,3-dihydrobenzofuran (**124**).

The most pronounced separation on PC 2 was the separation of Tomintoul (+ve) from Hobbister Hill samples (-ve). This component also separated, to a lesser degree, St Fergus (+ve) from Islay samples (-ve). Most compounds, with the exception of the nitrogen-containing compounds, had a positive effect on PC 2. In this way, Tomintoul and St Fergus samples on the positive side of PC 2 were found to contain relatively low concentrations of nitrogen-compounds compared to Hobbister Hill samples and Islay samples respectively. The carbohydrate derivatives and aromatics located on the negative side of PC 1 had particularly high positive values on PC 2 and were therefore particularly important for the separation of Tomintoul samples from Hobbister Hill samples.



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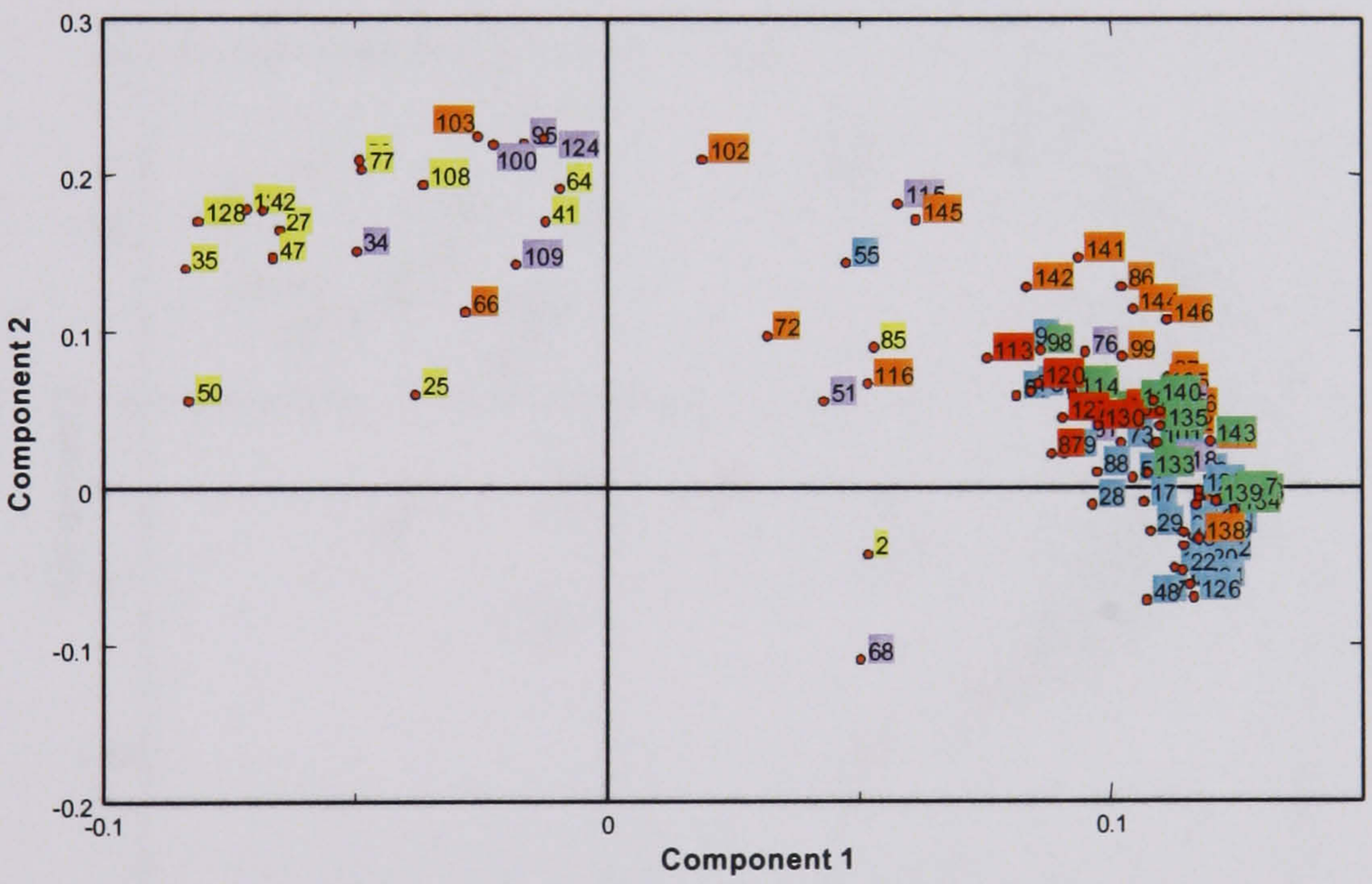

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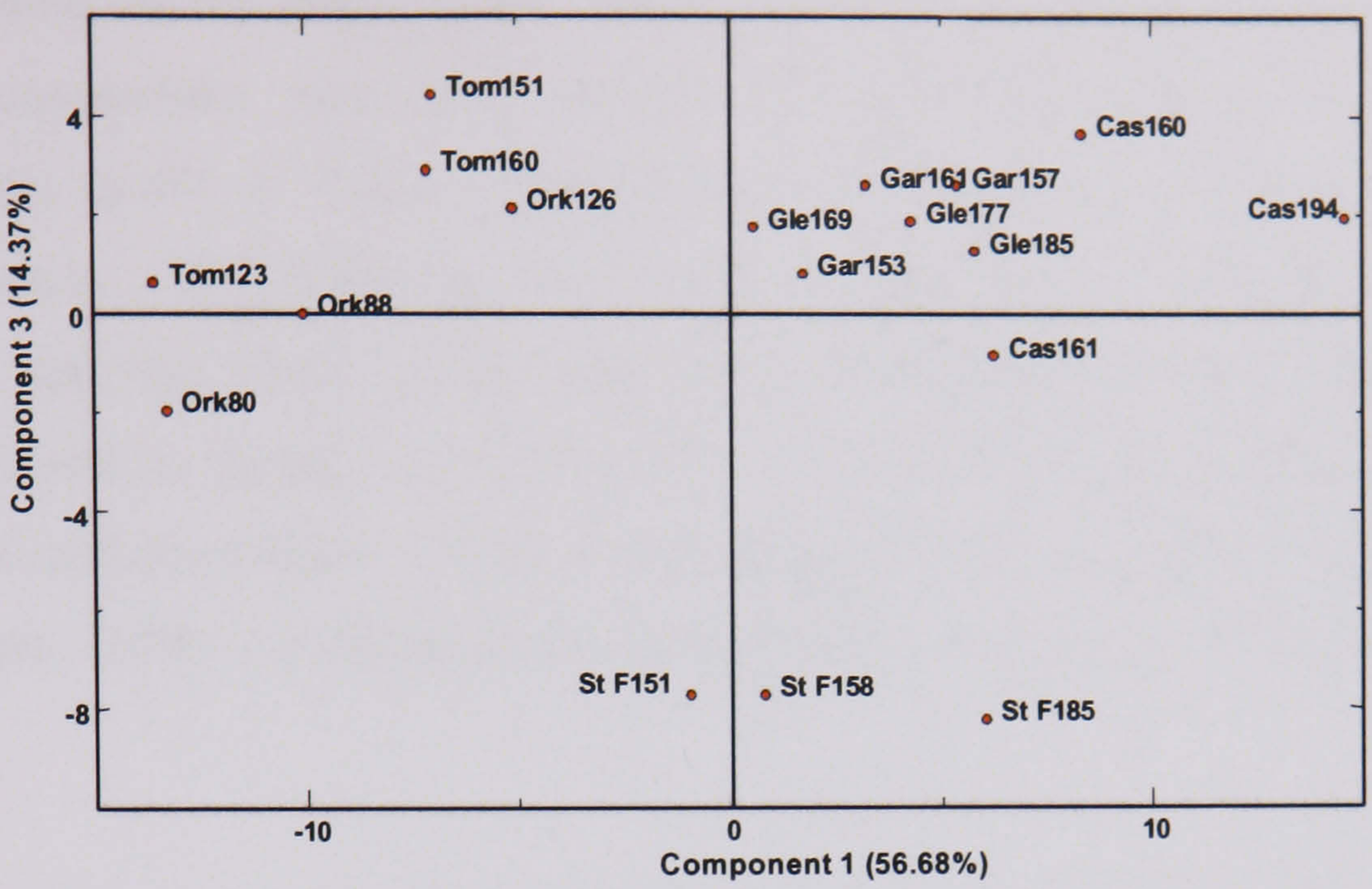
Figure 3.17. PCA of peat-derived compounds on lab-scale peated malts. a: Scores plot for PCs 1 and 2. Samples codes are described in Table 3.7 and samples are identified by their total marker phenols levels. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers as per Table 3.9 (Appendix E). Colour coding refers to compound classes defined in Table 3.9 (Appendix E).



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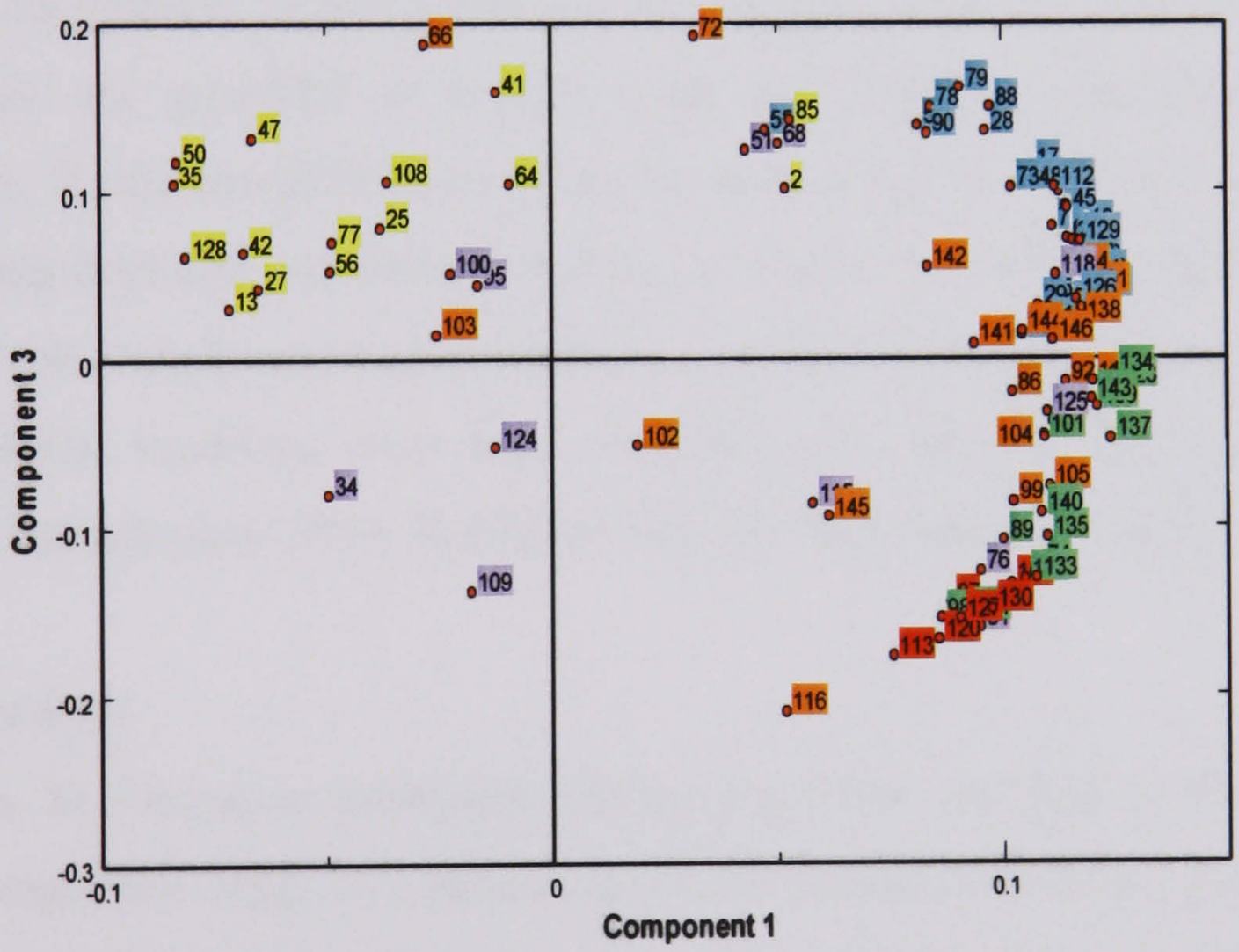

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Figure 3.18. PCA of peat-derived compounds on lab-scale peated malts. a: Scores plot for PCs 1 and 3. Samples codes are described in Table 3.7 and samples are identified by their total marker phenols levels. b: Loadings plot for PCs 1 and 3. Compounds are represented by their peak numbers as per Table 3.9 (Appendix E). Colour coding refers to compound classes defined in Table 3.9 (Appendix E).

The third principal component was found to separate samples made using the St Fergus peat (-ve) from the rest (Fig. 3.18a). The loadings for this PC showed that lignin-derived guaiacyl compounds and, most notably, syringyl compounds were particularly characteristic of the St Fergus samples (Fig. 3.18b). Some of the phenols also had negative values on PC 3 making them relatively abundant in St Fergus samples. Most prominent amongst these compounds was 4-butylphenol (**116**). Several aromatic compounds also had negative values on PC 3: two alkyl aryl ethers (dimethoxytoluene 1 (**61**) and dimethoxytoluene 2 (**76**)) and four benzofuran derivatives (benzofuran (**34**), dibenzofuran (**109**), 2,3-dihydrobenzofuran (**115**) and 2-methyl-2,3-dihydrobenzofuran (**124**)).

3.2.5 Comparison of chemical profiles of peat and lab-scale peated malts

Due to the differences in the particular compounds analysed and the instrumentation used, it was not possible to analyse peat and peated malt data simultaneously. Nevertheless, it was possible to compare the general patterns found using PCA for the two individual data sets. As shown in Figs. 3.17 and 3.18, when the peated malt data for all peat-derived compounds were analysed using PCA it was found that samples from the six geographical locations were well separated into the four clusters defined by the analysis of peat samples (Islay, Hobbister Hill, St Fergus and Tomintoul).

Lignin derivatives

Those peats, St Fergus in particular, containing relatively high levels of syringyl and guaiacyl compounds (Fig. 3.7) produced peated malts containing correspondingly high levels (Fig. 3.17 and 3.18). The slight separation of the different guaiacyl compounds more directly related to lignin (propenylguaiacol isomers) from that indicating lignin degradation (i.e. acetovanillone) was also maintained (Fig. 3.7 and Figs. 3.17 and 3.18).

Phenols

These non-specific phenolic compounds were less well clustered in both peat and peated malt than the lignin-derived syringols and guaiacols. Nevertheless, it was still possible to

see that Islay and St Fergus peats containing relatively high abundances of the majority of these compounds tended to produce malts containing high levels of the majority of these compounds (Figs. 3.7 and 3.18).

Carbohydrate-derived compounds

Generally, these compounds clustered together in peat and peated malt analyses (Figs. 3.7 and 3.17). As was found in peat samples, those samples with a relatively high abundance of these compounds, Hobbister Hill and Tomintoul, had a relatively low abundance of phenolic and nitrogen-containing compounds. Also, these compounds were particularly prevalent in Tomintoul samples in both peat and peated malt.

Nitrogen-containing compounds

There was found to be an increase in the number of nitrogen-containing species in peated malts compared with the raw peat. In peat samples, only nine nitrogen-containing compounds were detected whereas in peated malt 46 were detected. Despite the large difference in the numbers of nitrogen-containing compounds detected in peat and peated malt, their distribution could be correlated in the two matrices. In both peat and peated malt, Islay samples contained particularly high abundances of this group of compounds (Fig. 3.7 and Fig. 3.17).

Aromatics

This class comprised different types of compounds. The particular species found in peat and peated malt were different so it was not possible to directly relate the levels of this compound class as a whole in peat and peated malt. For example, the alkyl benzenes important for characterising Hobbister Hill peat were not detected in the peated malt analysis. Also, other aromatics which characterised Hobbister Hill peat, acetophenone, indene and methylindene, were not found to significantly differentiate peated malts when measured in absolute terms. That is not to say, however, that these compounds are not of relative importance in a particular malt.

One aromatic compound that was detected in both peat and peated malt and contributed significantly to differences between samples was 2-methyl-2,3-dihydrobenzofuran. In both peat and peated malt, this compound was found to most closely associate with the Tomintoul samples.

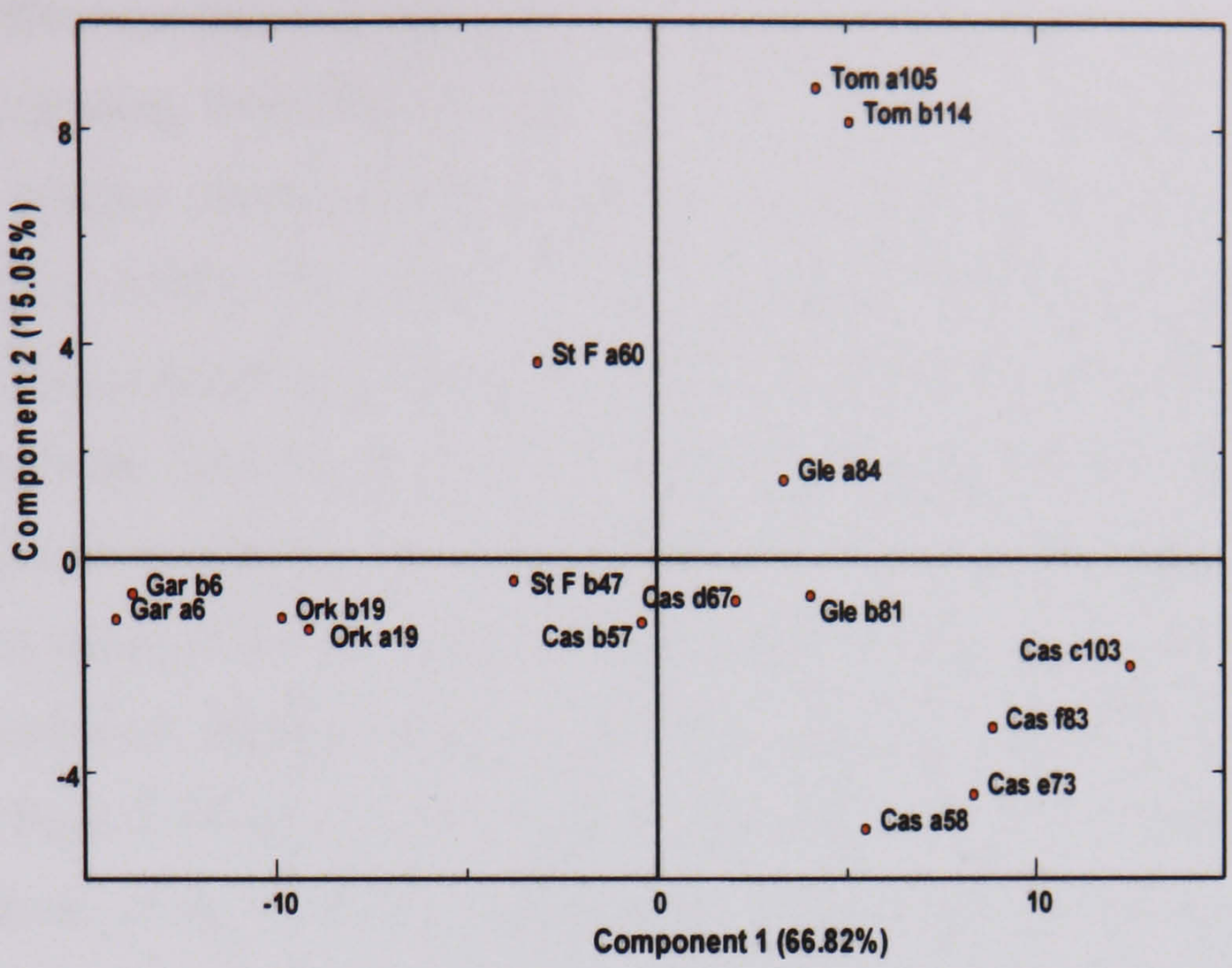
3.2.6 Total chemical profiles of industrially produced peated malts

Response ratio data for the industrial samples are presented in Table 3.12 (Appendix G). Ten compounds found in lab-scale samples were not readily identifiable in the industrial samples: 2-methylcyclopentanone (**2**), 2-cyclopenten-1-one (**13**), ethylpicoline 3 (**20**), lutidine 6 (**30**), 3, 4-dimethylcyclopentene-1-one (**39**), 2-methylbenzoxazole (**53**), methylfuranone (**56**), pyridinol (**121**), 2-methyl-2,3-dihydrobenzofuran (**124**) and unknown phenolic (**138**). Therefore, only 100 of the 108 peat-derived compounds that were found to significantly differentiate malts by peat site in the lab-scale samples were detected in the industrial samples. These 100 compounds were used for PCA of the industrial peated malts.

As the concentrations and proportions of peat-derived compounds in industrial and lab-scale peated malts were different (Tables 3.7 and 3.8 and Fig. 3.13), directly comparing the two data sets was difficult. Therefore, for this analysis, the industrial peated malts were analysed independently and the separation of samples was compared with that found for lab-scale peated malts. To relate the separation of samples in the two data sets, the five PCs with an eigen value of more than one (Table 3.13) in the industrial data set were explored to try and identify clustering similar to that found in the lab-scale samples. Only the first two components, explaining 81.87% of the total variance, were found to meet this criterion.

Table 3.13. Variance table for PCA of industrial peated malt samples.

Component No.	Eigen value	Percent variance	Cumulative % variance
1	66.82	66.82	66.82
2	15.05	15.05	81.87
3	8.16	8.16	90.03
4	4.29	4.29	94.33
5	1.96	1.96	96.28



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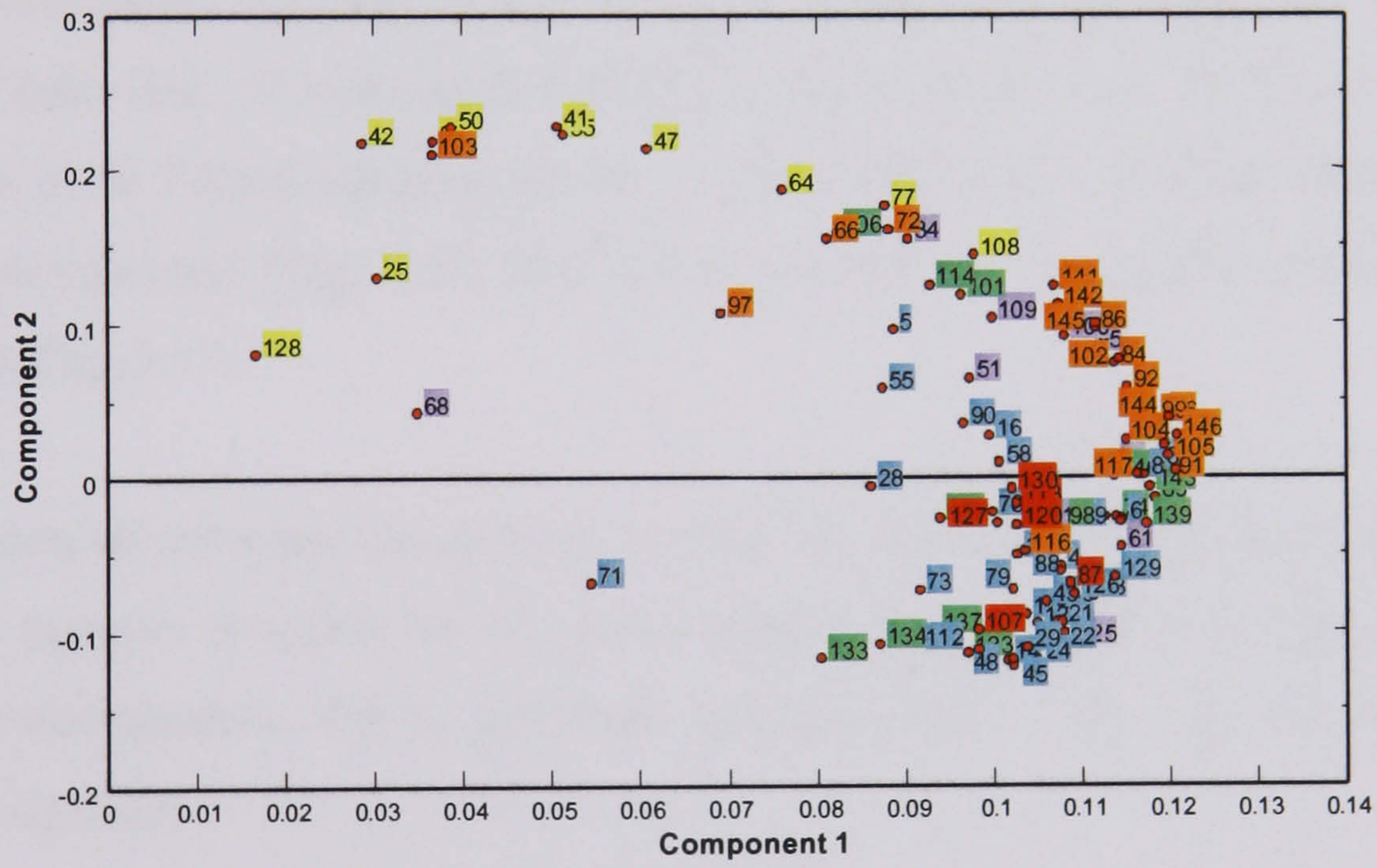


b

Figure 3.19. PCA of peat-derived compounds on industrial peated malts. a: Score plot for PCs 1 and 2. Sample codes are defined in Table 3.8. Total marker phenols concentrations are indicated for each sample. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers as per Table 3.9 (Appendix E). Colour coding refers to compound classes defined in Table 3.9 (Appendix E).

The major difference found in this data set, as indicated by PC 1, was generally due to differences in peating level (Fig. 3.19a). Moving from left to right along PC 1, samples with higher marker phenol levels generally had higher levels of all peat-derived compounds (Fig. 3.19b). However, it was noted that carbohydrate-derived compounds were less directly related to peating level than the other compounds (Fig. 3.19b). A similar finding was made in the case of the lab-scale samples where the carbohydrate-derived compound levels did not correlate with the levels of the other compounds (Fig. 3.17). Also in terms of carbohydrate derivatives, in both the industrial and lab-scale data sets, the Tomintoul samples were found to contain a relative abundance of these compounds (Figs. 3.17 and 3.19). Furthermore, the aromatic compounds (**34**, **95**, **100** and **109**) which, along with the carbohydrate compounds, characterised the lab-scale Tomintoul samples in Fig. 3.17 also characterised the industrial Tomintoul samples in Fig. 3.19. Additionally, a phenolic compound, C3 phenol 6 (**103**), also co-localised with these carbohydrate derivatives and aromatic compounds in both the lab-scale and industrial data sets. It was noted that in the industrial data set there was also a minor separation of St Fergus samples due to a relative abundance of these carbohydrate and aromatic compounds (Fig. 3.19) and this was similar to the pattern found for lab-scale samples in Fig. 3.17.

On analysing all principal components in the PCA of industrial samples, the separation of St Fergus samples found in the lab-scale samples due to a relatively high abundance of lignin-derived phenols, and in particular syringols (Fig. 3.18), was not apparent in the industrial samples.

There was some similarity between lab-scale and industrial samples in terms of the proportion of oxygenated guaiacyl compounds present. In both data sets it was found that samples which contained relatively high levels of these compounds (vanillic acid methyl ester (**133**), acetovanillone (**134**) and guaiacyl propanaldehyde (**137**)) contained relatively low abundances of guaiacyl compounds with propenyl side chains (eugenol (**98**), propenylmethoxyphenol 1 (**106**), propenylmethoxyphenol 2 (**110**) and propenylmethoxyphenol 3 (**114**)) (Figs. 3.17, 3.18 and 3.19). Amongst both the lab-scale

and industrial samples it was noted that the island samples (Islay and Hobbister Hill) tended to have a larger proportion of oxygenated guaiacols whilst the mainland samples (St Fergus and Tomintoul) contained a larger proportion of propenyl guaiacols. Also in both sample sets, those samples containing a higher proportion of oxygenated guaiacols also contained a relatively high proportion of the nitrogen-containing compounds.

3.2.7 Summary

Analysis of lab-scale peated malt using HS-SPME and SPE in combination with GC-MS showed that the classes of compounds detected in the pyrolysates of peat were passed to malt during kilning. These compounds were used to differentiate peated malts according to peat source in a pattern similar to that found in the peat itself. Differentiation due to peat source was also detectable using only the marker phenols commonly used to determine peating level. In addition, several aspects of this differentiation were detected in industrially produced malt.

3.3 Peated New-Make Spirit

3.3.1 Introduction

The composition of peated malt was found to be influenced by the source of the peat used in its production. The next stage of this research was therefore to determine whether this influence could be detected in new-make spirit. In this regard, lab-scale peated new-make spirits were produced from the lab-scale malts peated using peat from the six sampled geographical locations. Lab-scale studies eliminated the production variations which influence the composition of industrially produced new-make spirits. The composition of the new-make spirits were analysed and correlated with that of the peated malt and indeed the peat itself. Also, industrially produced peated new-make spirits were analysed to see how much of an impact industrial variations have on any differentiation due to peat source. Finally, the influence of peat composition on flavour was then determined by use of sensory analysis, the results of which could be correlated with analytical data to indicate which compounds contribute to the peat-derived flavour of new-make spirit.

3.3.2 Marker phenols profiles of lab-scale new-make spirits

Lab-scale new-make spirit samples were produced using lab-scale peated malt which in turn had been produced using peat from each of the six sampled peat sources. These samples were then analysed by directly injecting them into the GC-MS as described in Chapter 2.8. A typical chromatogram is shown in Fig. 3.20. The marker phenols concentrations of these samples are shown in Table 3.14.

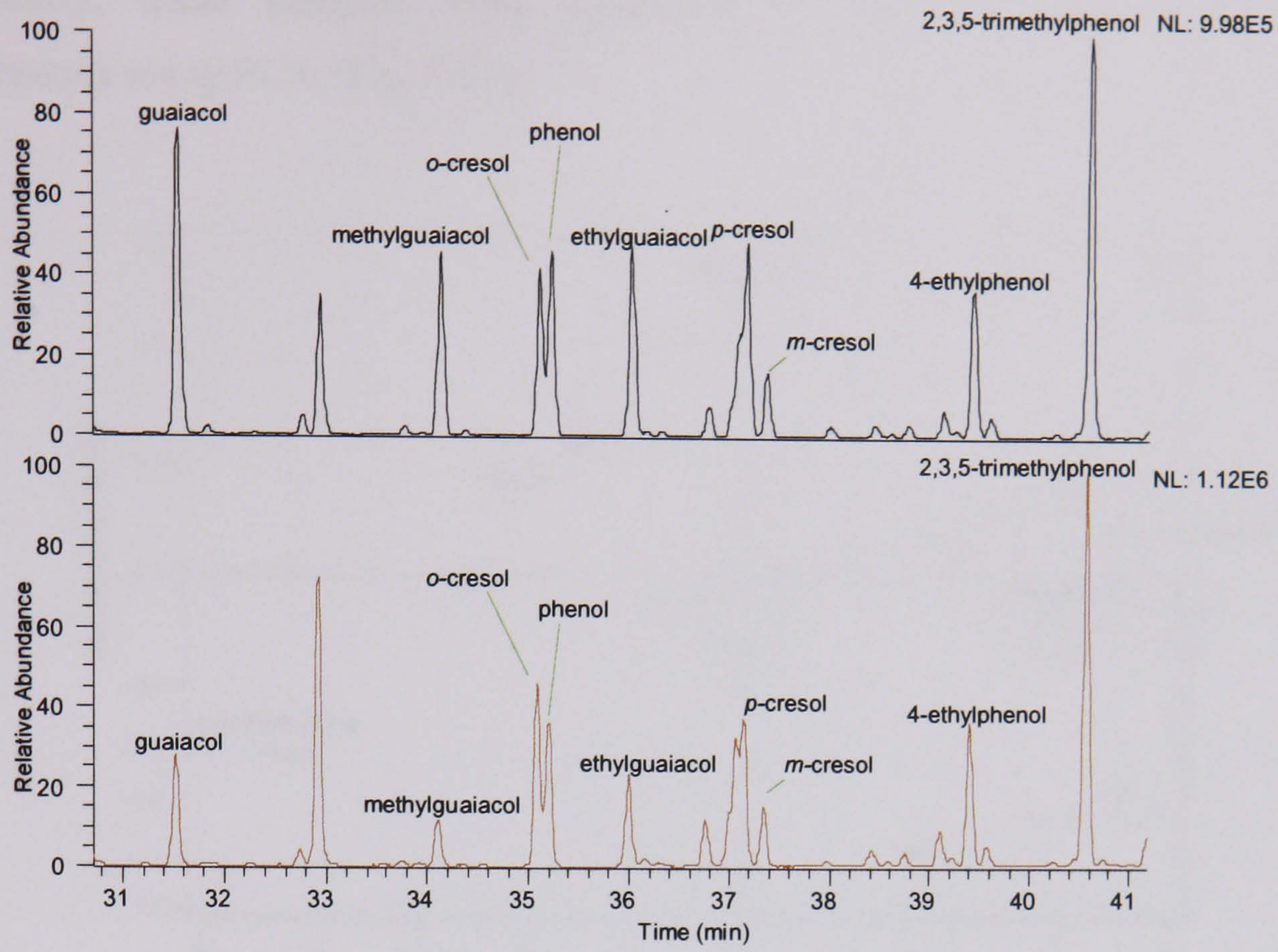
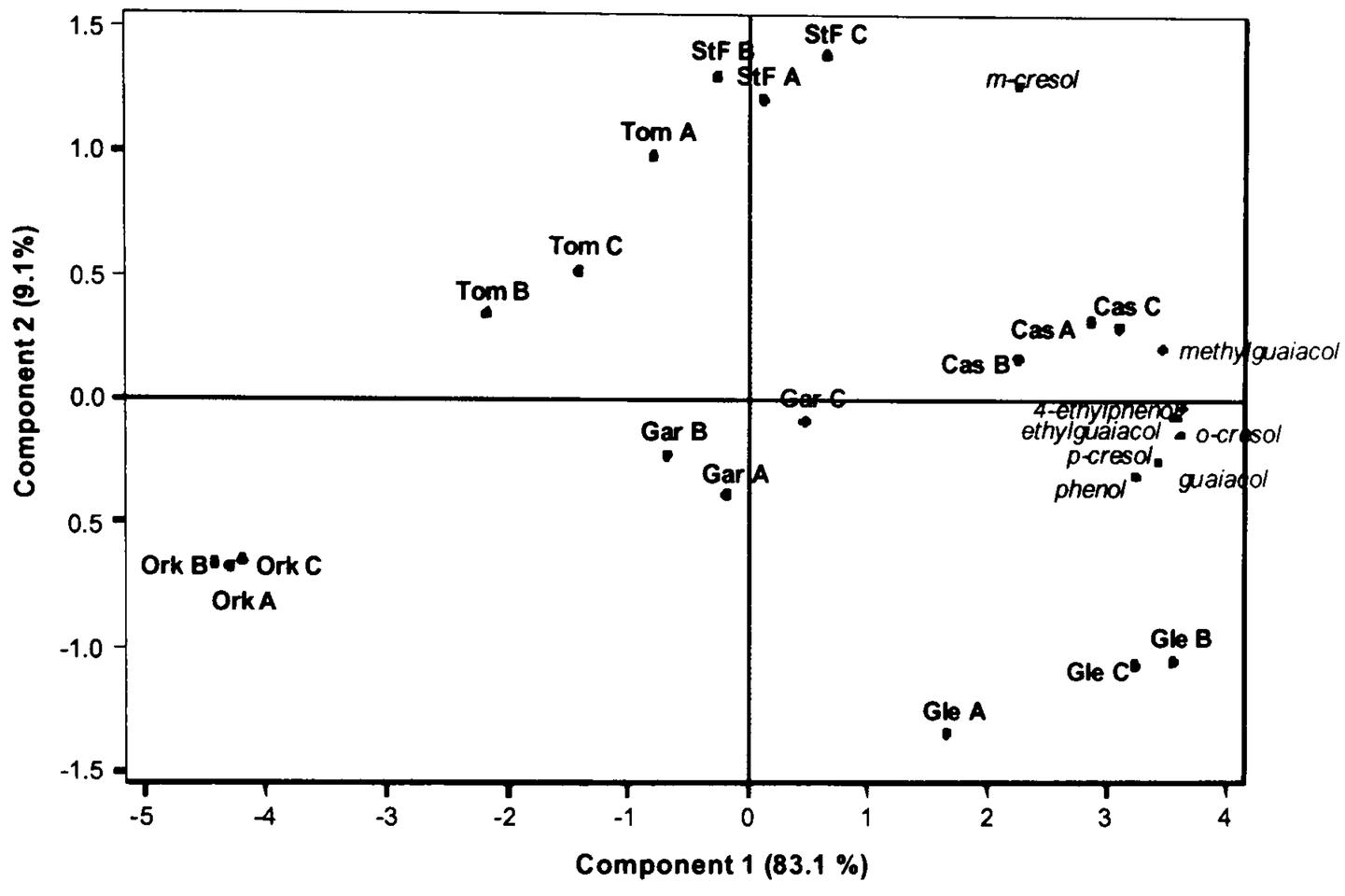


Fig. 3.20. Chromatogram produced from direct injection-GC-MS analysis of new-make spirit. M/z shown = 93.50–94.50, 106.50–107.50, 107.50–108.50, 108.50–109.50, 120.50–121.50, 121.50–122.50, 122.50–123.50, 123.50–124.50, 135.50–136.50, 136.50–137.50, 137.50–138.50 and 151.50–152.50. Black chromatograph is lab-scale spirit, red chromatograph is industrial spirit (both produced using Castlehill peat).

Table 3.14. Concentrations (ppm) of marker phenols in peated new-make spirit samples. Number of samples per peat source = 3. Figures in brackets are % RSD.

Sample	Guaiacol	Methylguaiacol	*o*-Cresol	Phenol	Ethylguaiacol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	Total phenols
Castlehill (Cas)	10.0 (2.5)	6.5 (3.9)	4.6 (2.8)	7.2 (2.8)	4.1 (3.8)	4.4 (2.6)	1.9 (2.3)	2.5 (3.3)	41.1 (2.7)
Gartbreck (Gar)	7.9 (7.2)	4.7 (6.1)	4.2 (6.0)	6.7 (6.1)	2.9 (5.6)	3.7 (5.4)	1.7 (4.6)	2.0 (4.8)	33.8 (4.7)
Glenmachrie (Gle)	9.9 (4.5)	6.4 (7.4)	4.7 (4.7)	8.4 (5.3)	3.9 (6.9)	4.7 (5.1)	1.6 (5.0)	2.4 (6.0)	42.0 (5.4)
Hobbister (Ork)	4.9 (2.3)	3.1 (3.5)	3.2 (2.9)	4.8 (2.3)	2.0 (2.8)	2.5 (2.0)	1.3 (1.9)	1.5 (1.4)	23.4 (1.6)
St Fergus (StF)	7.7 (5.8)	6.0 (6.5)	4.0 (4.0)	6.1 (3.9)	3.2 (5.5)	3.5 (2.3)	2.0 (2.0)	2.0 (3.3)	34.5 (4.2)
Tomintoul (Tom)	4.8 (3.6)	3.7 (9.0)	4.0 (2.1)	6.8 (6.7)	2.3 (7.1)	3.6 (5.7)	1.8 (5.8)	1.9 (5.0)	28.8 (5.0)

Subsequently, these samples were compared in terms of their marker phenols concentrations using PCA (Fig. 3.21).



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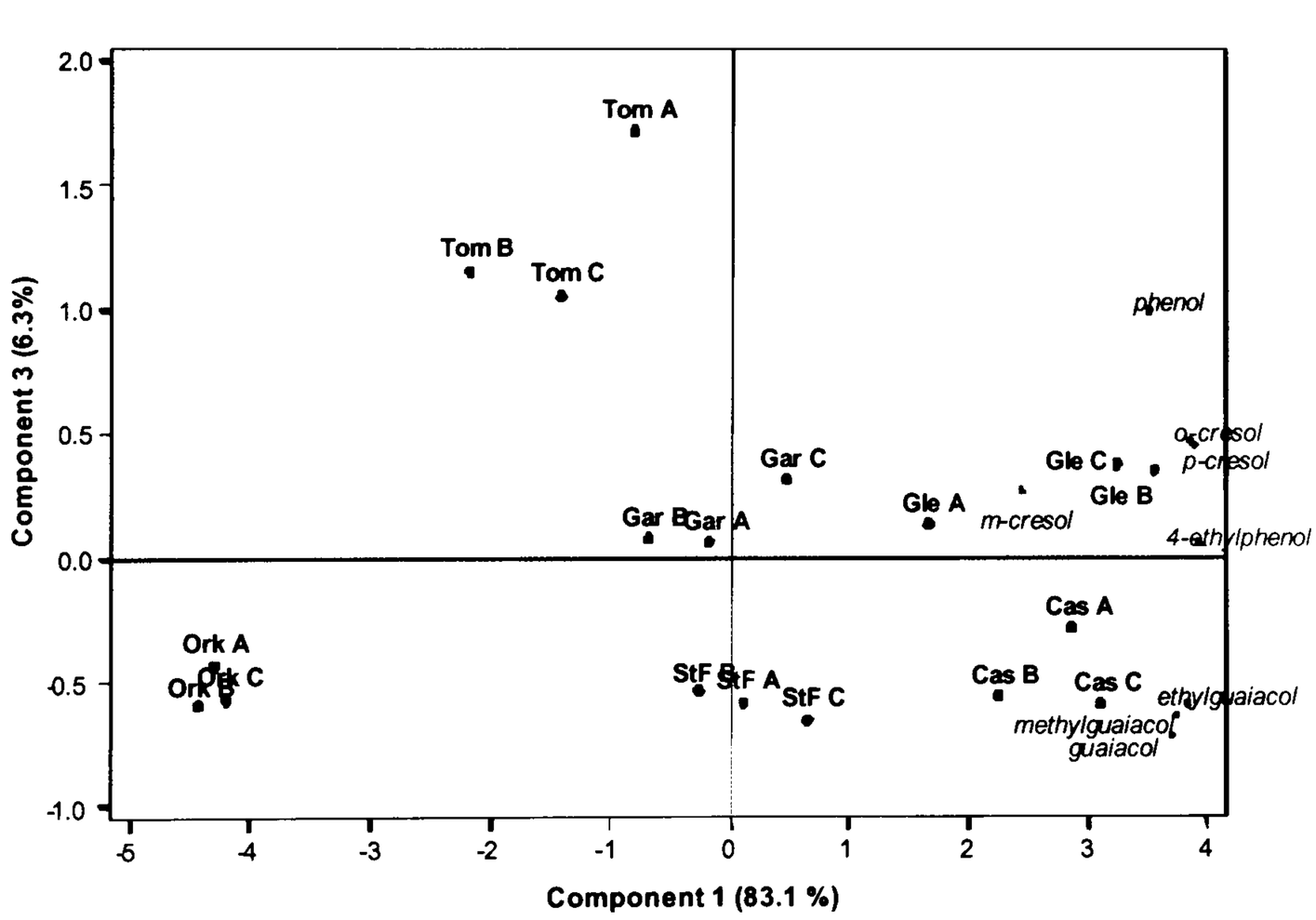

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Figure 3.21. PCA of marker phenol concentrations in lab-scale peated new-make spirits. Sample codes are defined in Table 3.14. a: Bi plot of PCA scores and loadings for PCs 1 and 2. b: Bi plot of PCA scores and loadings for PCs 1 and 3.

Fig. 3.21a shows that PC 1 explains a large majority of the total variance (83.1%). This PC relates to the total concentration of marker phenols. Despite the peating levels used in the production of the lab-scale spirits being the same, samples produced using Glenmachrie and Castlehill peat contained large total concentrations of marker phenols whilst Hobbister Hill samples contained relatively low levels. *m*-Cresol did not contribute to this component as much as the rest. Rather, this compound had a positive influence on PC 2 and was thus found in particularly high abundance, along with methylguaiacol, in the St Fergus samples.

PC 3 described a difference in the ratio of guaiacols to other phenols (Fig. 3.21b). This component showed that Tomintoul samples had a relatively low proportion of guaiacols and a correspondingly high proportion of phenols.

3.3.3 Marker phenols profiles of industrial new-make spirits

Using the same method as was used for the lab-scale spirits, the marker phenols levels for a range of industrially produced new-make spirit samples were measured (Table 3.15).

Table 3.15. Marker phenols concentrations (ppm) in a range of industrial peated new-make spirits.

Sample	Guaiacol	Methylguaiacol	*o*-Cresol	Phenol	Ethylguaiacol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	Total phenols
Islay a	4.5	2.0	6.4	7.2	2.1	5.9	2.2	3.4	33.6
Islay b	3.5	1.7	5.2	5.3	1.6	4.5	1.6	2.3	25.7
Islay c	4.1	2.0	5.7	6.2	2.2	5.3	1.9	2.8	30.2
Islay d	3.2	1.6	4.1	4.7	1.6	3.7	1.4	1.9	22.3
Islay e	2.5	1.4	4.0	4.6	1.4	4.4	1.5	2.5	22.2
Islay f	1.0	0.6	1.2	1.4	0.5	1.1	0.6	0.6	7.0
Orkney a	0.5	0.5	0.6	0.4	0.4	0.5	0.3	0.3	3.4
Orkney b	0.4	0.4	0.5	0.3	0.4	0.4	0.3	0.2	2.9
St F a	3.3	2.3	5.1	6.0	2.1	4.5	2.0	2.1	27.3
St F b	1.3	1.1	2.3	2.7	0.9	2.1	1.0	1.1	12.6
St F f	2.8	1.5	4.4	4.5	1.4	3.7	1.5	1.8	21.6
St F g	2.2	1.6	3.8	4.4	1.3	3.6	1.6	1.7	20.1

As with the peated malt samples, different relative proportions of marker phenols were detected in the industrial and lab-scale spirit samples, with the lab-scale samples containing a relatively high proportion of guaiacols (Fig. 3.22). This was because, in absolute terms, the lab-scale spirits tended to contain higher levels of the guaiacols (Tables 3.14 and 3.15). The levels of the other marker phenols in the more highly peated industrial spirits were similar to those found in the lab-scale samples.

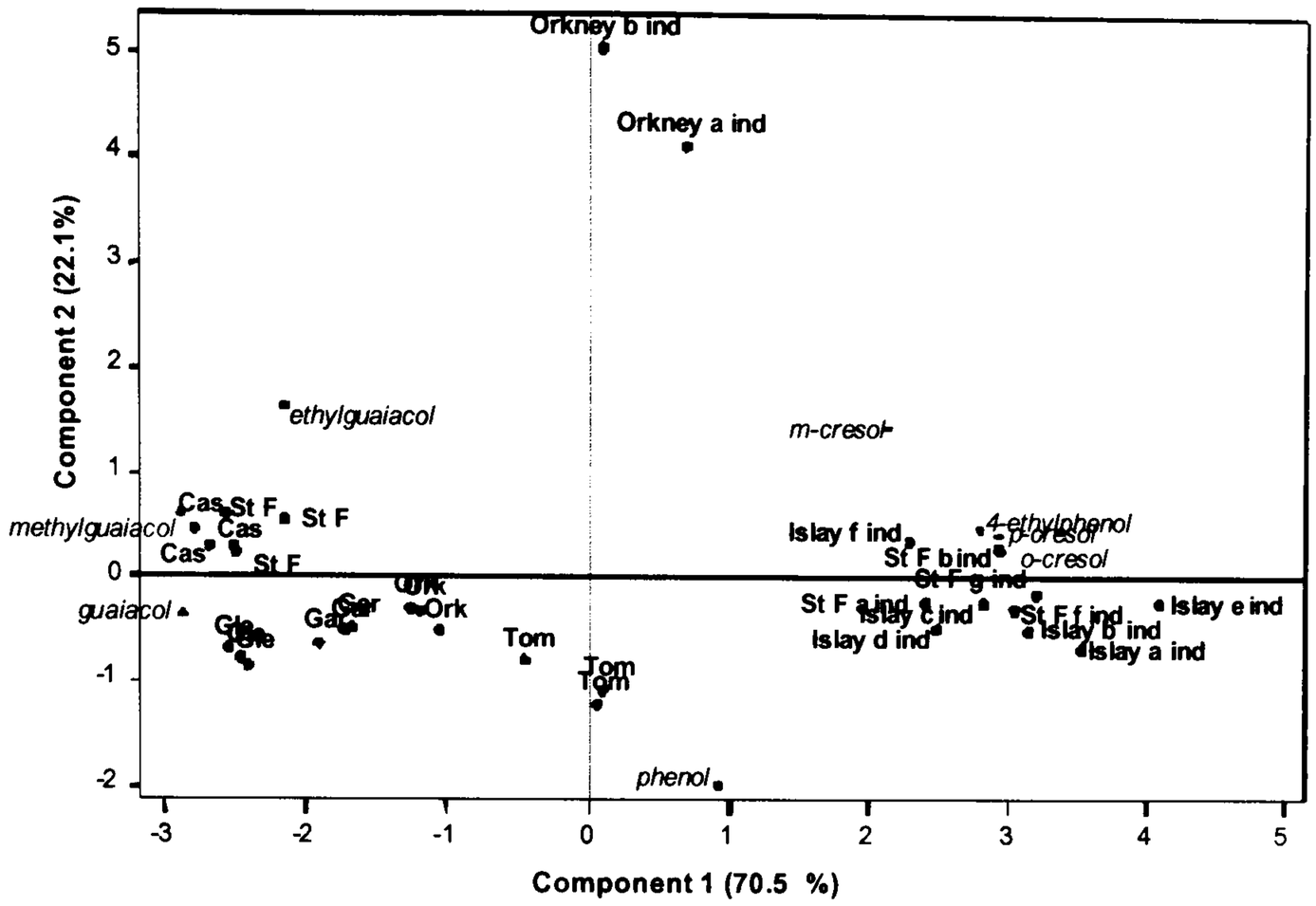


Figure 3.22. PCA bi plot of normalised marker phenols concentrations in industrial and lab-scale peated spirit samples. Industrial samples are labelled "ind".

PCA was used to compare the levels of the marker phenols in the various industrial samples and any patterns were related to those found in lab-scale samples (Fig. 3.23). Clearly, PC 1 relates to the overall level of compounds found in each spirit. Explaining 97.40% of variance, this was found to be a much more important source of variance in industrial spirit than in lab-scale spirit (where PC 1 explained only 82.71% of variance in Fig. 3.21). This would be expected given that the amount of peat used to produce a spirit is not consistent across all industrial producers.

As a consequence of the large amount of variance being explained by peating level, subsequent components only explained very low levels of variance. Nevertheless, as shown in Fig. 3.23, PC 2 was able to separate Islay spirits from St Fergus spirits. It was even possible to distinguish between spirits produced in the same distillery using peat from different sources (highlighted green on Fig. 3.23). This separation was found to be due to a relative abundance of methylguaiacol in the St Fergus spirits whilst the Islay spirits were characterised by a relative abundance of 4-ethylphenol. Also, as with the

lab-scale equivalents, *m*-cresol was relatively abundant in the industrial St Fergus samples, though not as significant as methylguaiacol. It was additionally noted that whilst it was not as significant as *m*-cresol, methylguaiacol was also relatively abundant in the lab-scale St Fergus samples (Fig. 3.20a).

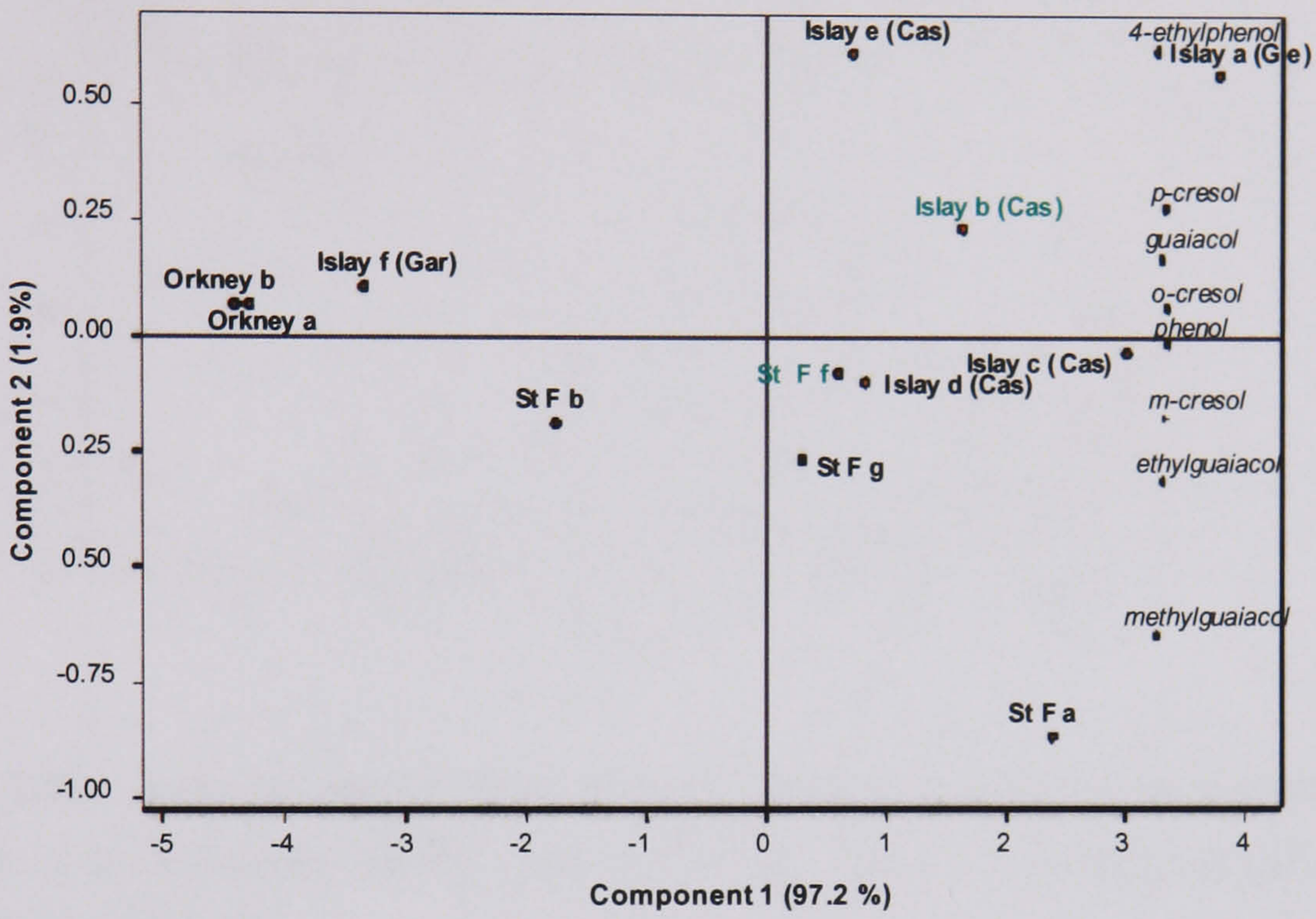


Figure 3.23. PCA bi plot of marker phenol concentrations in industrial peated new-make spirits. Sample codes are defined in Table 3.15.

3.3.4 Total chemical profiles of lab-scale new-make spirits

The lab-scale spirits were compared in terms of as many peat-derived compounds belonging to previously described classes (carbohydrate derivatives, guaiacols, syringols, phenols, nitrogen-containing compounds and aromatic compounds) as could be detected using the same method as was used for the analysis of the marker phenols (described in Chapter 2.8). Some unknown compounds were also included in this analysis given their structural similarities to known peat-derived compounds as defined by their mass spectra. A typical chromatogram of a peated new-make spirit is shown in Fig. 3.24.

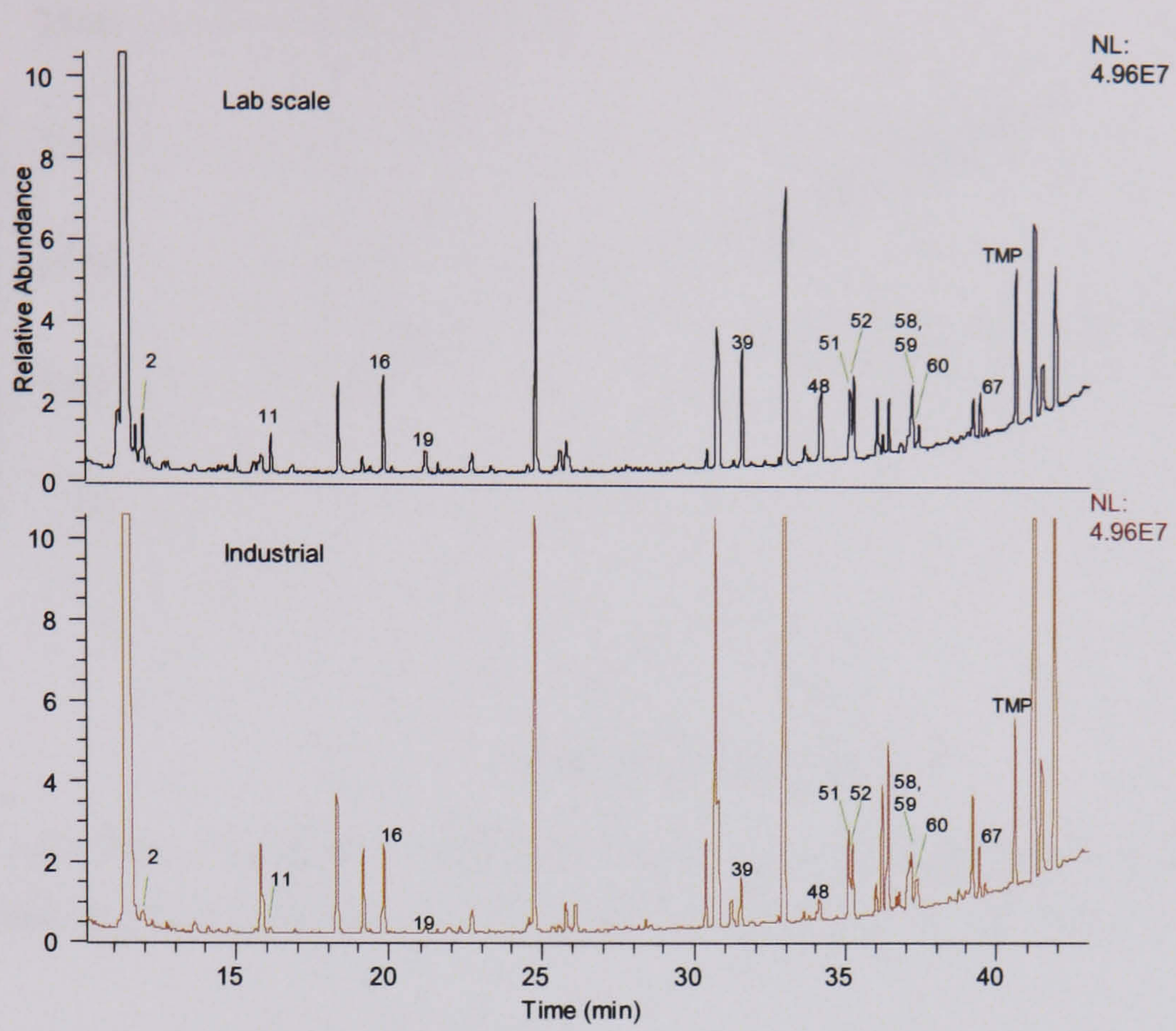


Figure 3.24. Total ion current chromatograms obtained from direct injection-GC-MS analysis of lab-scale and industrial peated new-make spirits. Both samples were made using the Castlehill peat.

Analysis of an unpeated malt allowed the identification of any compounds which were not derived from peat resulting in the identification of 72 peat-derived compounds (Table 3.16 (Appendix H)). The estimated retention indices listed in Table 3.16 were calculated as described in Chapter 2.4.2 and Fig. 3.25 shows the plot of literature retention index data for compounds identified here with a high degree of certainty by MS versus experimental retention time data.

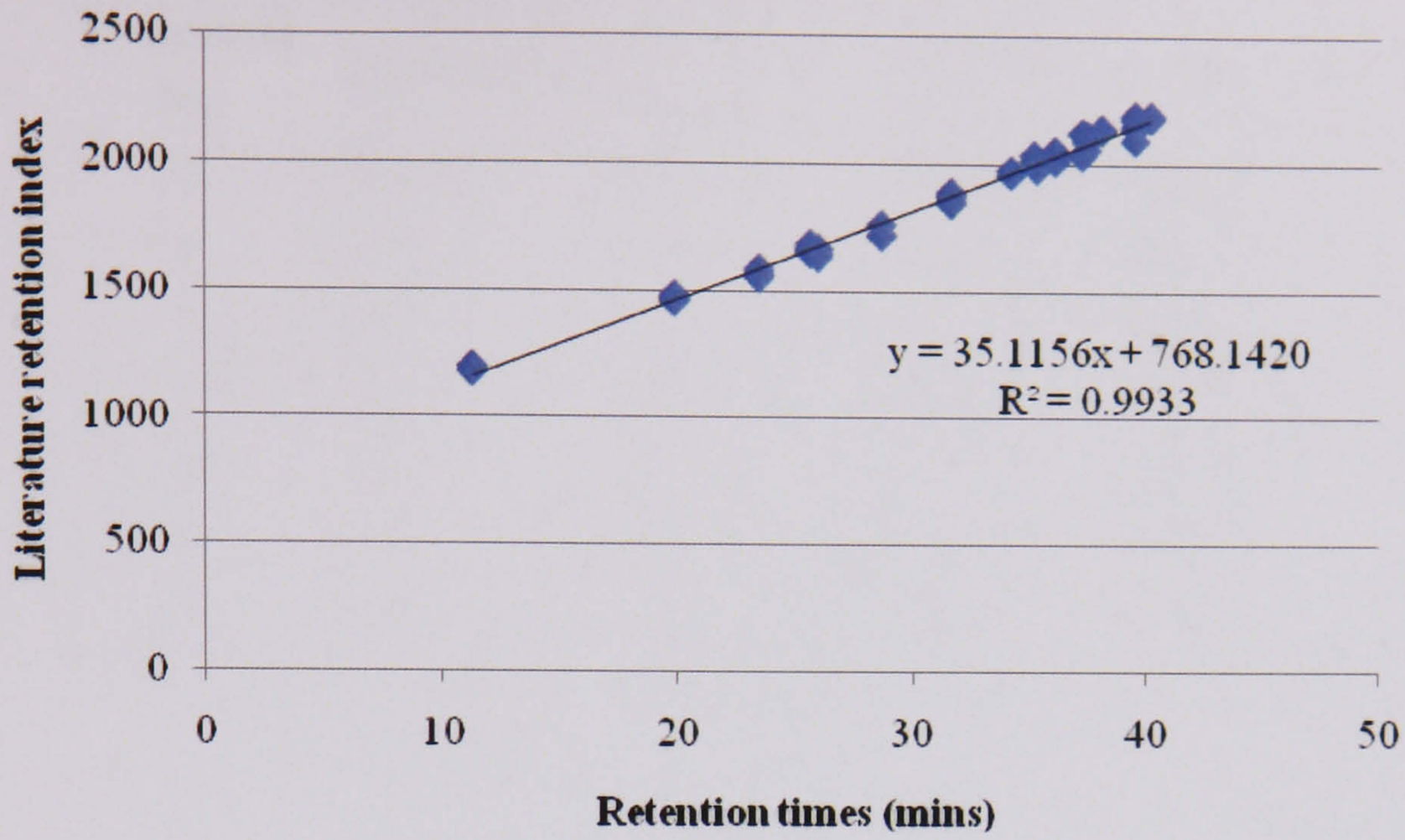


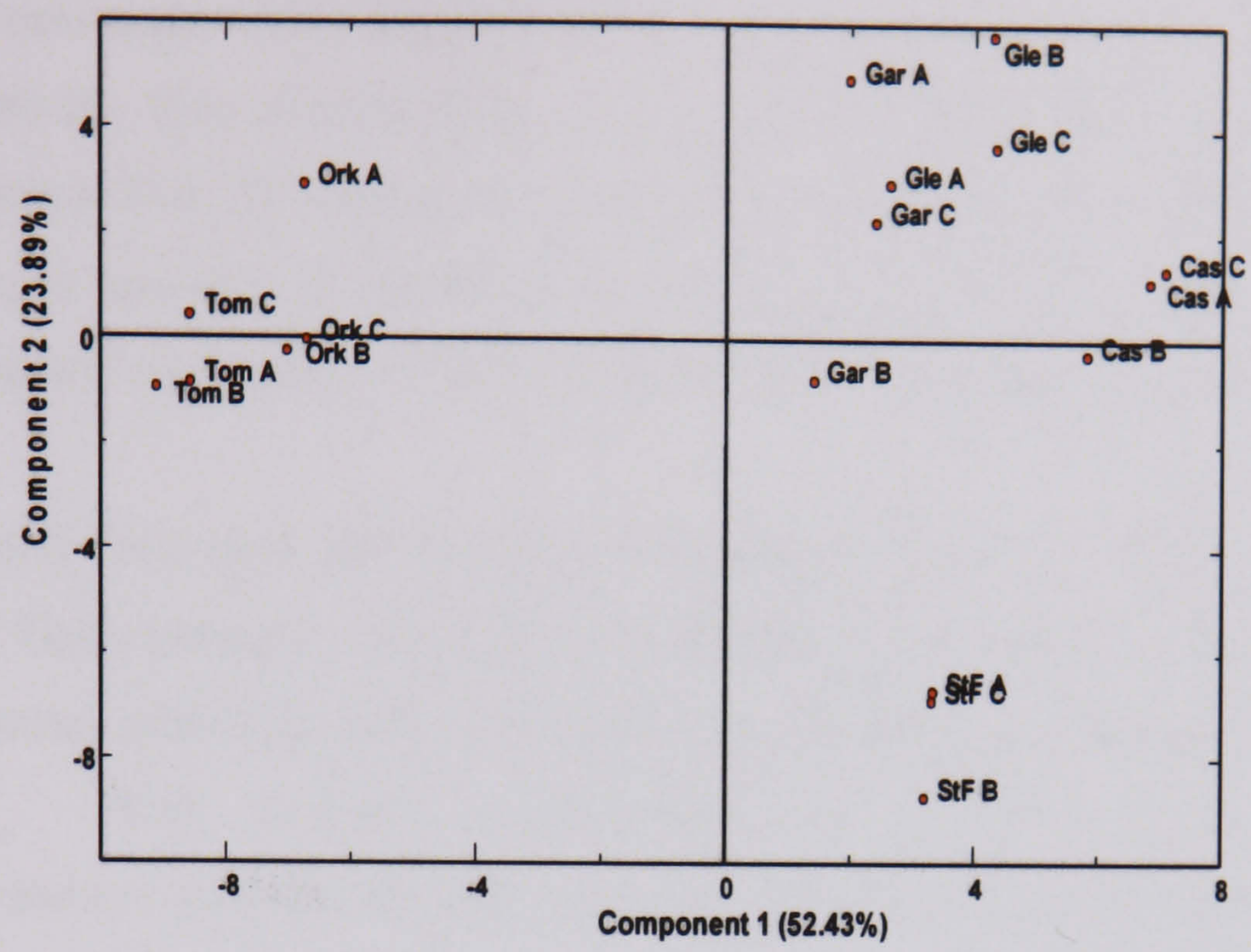
Figure 3.25. Plot of literature retention index data for compounds identified in new-make spirit with a high degree of certainty by MS versus experimental retention time data.

The abundances of these compounds in spirit produced from the six sampled peat locations are shown in Table 3.17 (Appendix I). ANOVA was then carried out to compare the levels of the 72 peat-derived compounds in the different peated new-make spirits and any compounds showing no significant difference between locations were removed (Table 3.17 (Appendix I)).

Using data for the remaining 66 compounds, PCA was carried out to identify any patterns in the data. Only those principal components with an eigen value of over 1 were considered. ANOVA was applied to the seven components which met this criterion to determine which components were statistically important in terms of differentiating samples made using peat from the six different sources (Table 3.18). This analysis showed that the first five components significantly differentiated between samples made using different peat. However, PCs 4 and 5 explained only relatively small amounts of variance and so only PCs 1 to 3 are considered here.

Table 3.18. Variance table for PCA of peated new-make spirit data.

Component No.	Eigen value	Percent variance	Cumulative % variance	p values
1	34.60	52.43	52.43	0.0000
2	15.77	23.89	76.32	0.0000
3	9.34	14.16	90.47	0.0000
4	1.65	2.51	92.98	0.0006
5	1.18	1.79	94.77	0.0031
6	1.11	1.68	96.45	0.5347
7	1.00	1.52	97.97	0.9323



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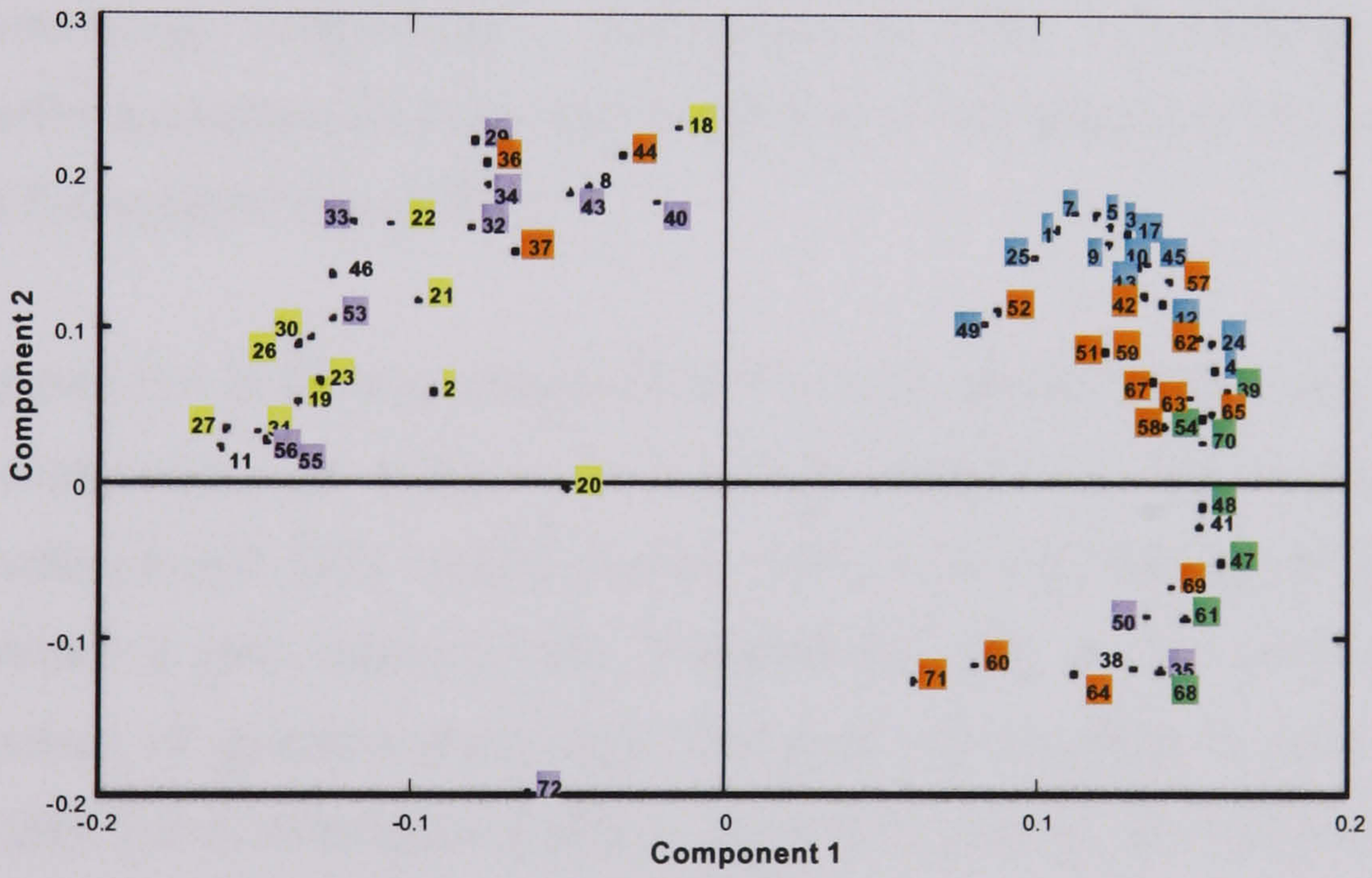


b

Figure 3.26. PCA of GC-MS data for lab-scale peated new-make spirits. a: Score plot for PCs 1 and 2. Sample codes are described in Table 3.14. b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers as per Table 3.16 (Appendix H). Colour coding refers to the compound classes defined in Table 3.16 (Appendix H).

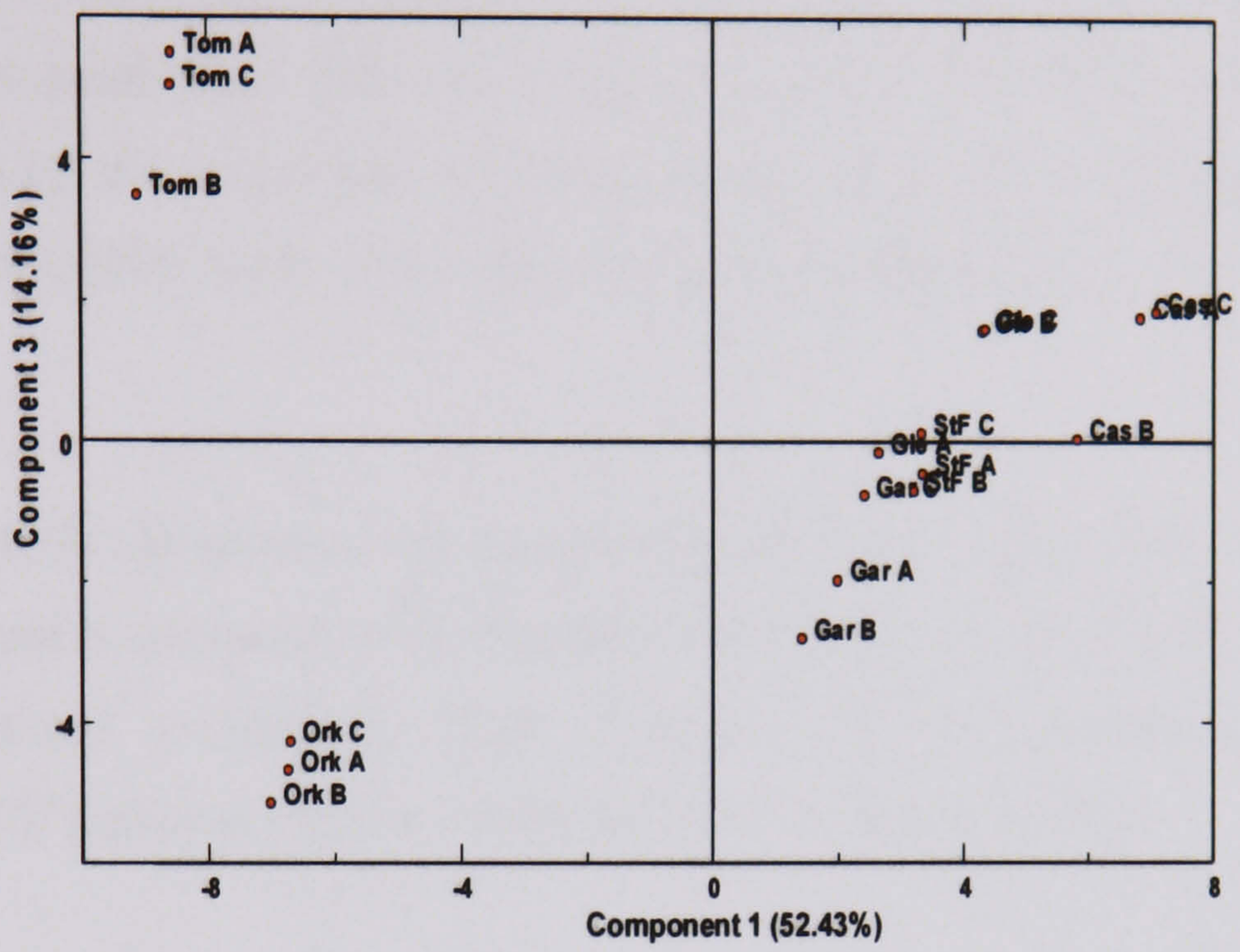
As shown in Figs. 3.26 and 3.27, when the data for peat-derived compounds were analysed in new-make spirit, it was found that samples from the six peat locations were separated into the four clusters defined by the analysis of peat samples in Chapter 3.1 (Islay, Hobbister Hill, St Fergus and Tomintoul). There was additionally found to be a relatively small amount of separation of new-make spirits made using the three Islay peats, particularly of Castlehill from Gartbreck and Glenmachrie (Fig. 3.26).

PC 1 separated Tomintoul and Hobbister Hill samples from Islay and St Fergus samples (Fig. 3.26). Those samples with negative values for PC 1 (Tomintoul and Hobbister Hill) tended to contain relatively high concentrations of carbohydrate derivatives and aromatic compounds. Two aromatic compounds were exceptions to this finding, dimethoxytoluene 1 (**35**) and dimethoxytoluene 2 (**50**), as these compounds were located on the positive side of PC 1. Those samples with positive values for PC 1 (St Fergus and Islay) tended to contain relatively high concentrations of lignin derivatives, phenols and nitrogen-containing compounds. 4-Acetylphenol (**36**), 2-chlorophenol (**37**) and hydroxymethylacetophenone (**44**) were exceptions to this finding as these were phenols located on the negative side of PC 1.

PC 2 separated the St Fergus samples from the Islay samples (Fig. 3.26). A subset of phenolic compounds was found to be relatively abundant in the St Fergus samples: methoxymethylphenol (**47**), methylguaiacol (**48**), *m*-cresol (**60**), propylguaiacol (**61**), dimethylphenol 2 (**64**), eugenol (**68**), 3-ethylphenol (**69**) and C3 phenol 5 (**71**). A different subset of phenolic compounds was relatively abundant in the Islay samples: guaiacols (**39**), 2,6-dimethylphenol (**42**), *o*-cresol (**51**), phenol (**52**), ethylguaiacol (**54**), 2-ethylphenol (**57**), dimethylphenol x2 (**58**), *p*-cresol (**59**), C3 phenol 1 (**62**), C3 phenol 2 (**63**), C3 phenol 3 (**65**), 4-ethylphenol (**67**) and vinylguaiacol (**70**). Additionally, all the nitrogen-containing compounds detected were relatively abundant in the Islay samples.

A group of other aromatic compounds were also relatively abundant in the St Fergus samples: dimethoxytoluene 1 (**35**), dimethoxytoluene 2 (**50**) and dibenzofuran (**72**). Two unknowns were also included in this group, unknown (**38**), unknown (**41**), as their mass

spectra were similar to those of the known aromatics. Other than these compounds and the previously mentioned phenols, all other compounds were found in relatively low abundances in the St Fergus samples. Compounds with particularly low abundances in St Fergus samples tended to be those located in the upper left quadrant of the PCA plot.



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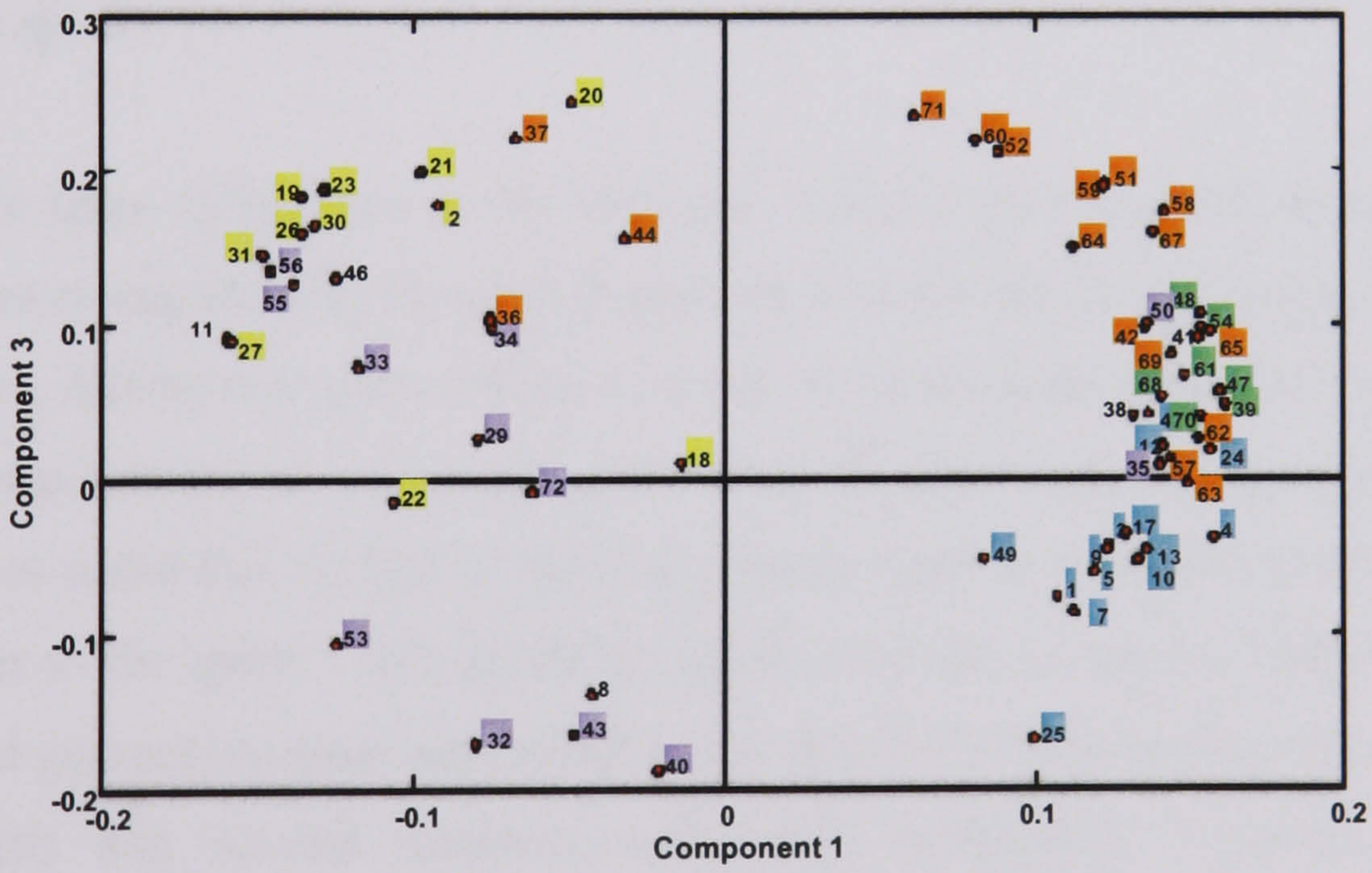


b

Figure 3.27. PCA of GC-MS data for lab-scale peated new-make spirits. a: Score plot for PCs 1 and 3. Sample codes are described in Table 3.14. b: Loadings plot for PCs 1 and 3. Compounds are represented by their peak numbers as per Table 3.16 (Appendix H). Colour coding refers to compound classes defined in Table 3.16 (Appendix H).

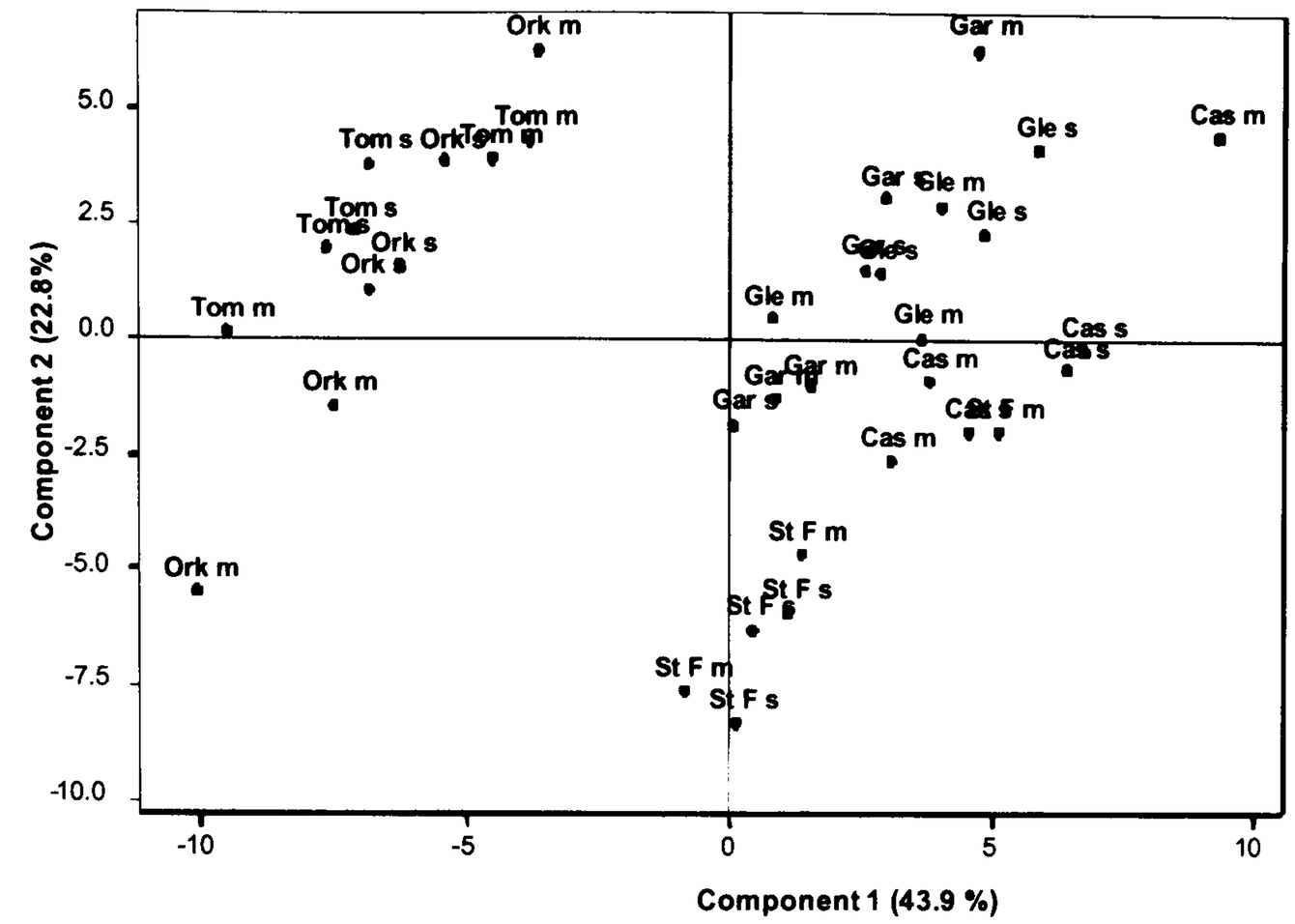
PC 3, as shown in Fig. 3.27, separated Tomintoul samples on the positive side from Hobbister Hill samples on the negative side. The following group of compounds were particularly characteristic of the Hobbister Hill samples having particularly high negative values for PC 3: unknown (**8**), benzonitrile (**25**), naphthalene (**32**), 2-methylnaphthalene (**40**), 1-methylnaphthalene (**43**) and biphenyl (**53**). The other nitrogen-containing compounds (with the exceptions of ethylmethylpyrazine (**12**), 3-methoxypyridine (**24**) and benzylnitrile (**45**)) were, to a lesser extent, also characteristic of the Hobbister Hill samples.

A relatively high abundance of carbohydrate-derived compounds characterised the Tomintoul samples compared with Hobbister Hill samples (Fig. 3.27). Also, Tomintoul samples contained a relatively high abundance of the phenols, most notably 2-chlorophenol (**37**), phenol (**52**), *m*-cresol (**60**) and C3 phenol 5 (**71**).

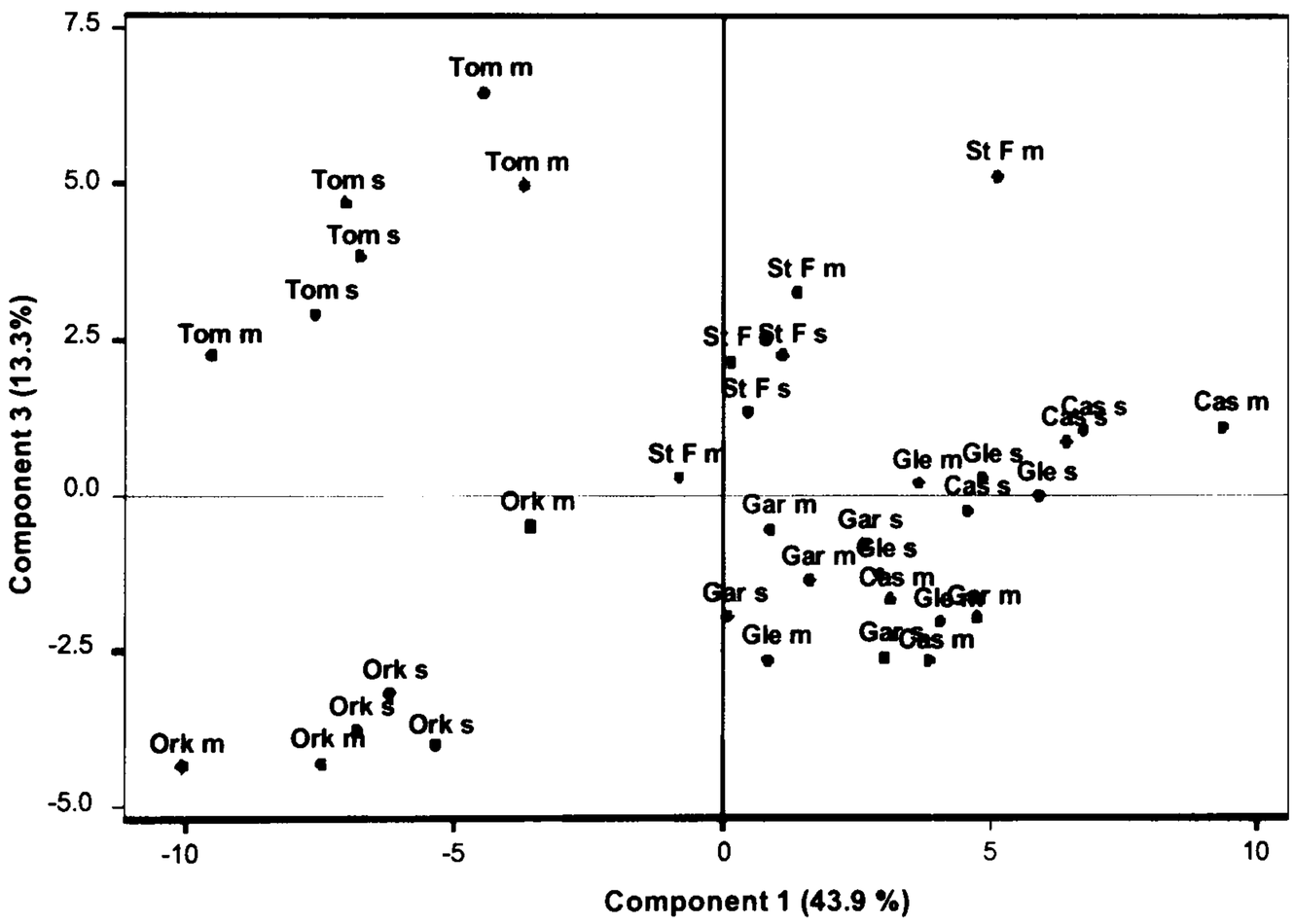
3.3.5 Comparison of chemical profiles of lab-scale peated malt and lab-scale peated new-make spirit

There were large differences in the absolute levels of peat-derived compounds in malt and spirit as exemplified by the marker phenols levels in the two matrices (Tables 3.7 and 3.14). Also, differences were evident in terms of the specific compounds present. There were fewer peat-derived compounds detected in the spirit, 72 compared with 146 in the malt. It was noted that several of the larger lignin-derived compounds found in malt did not transfer to the spirit. This group included all of the syringyl compounds, all of the oxygenated guaiacyl compounds (vanillin was detected in both peated and unpeated new-make spirit) and several guaiacyl compounds containing 3 carbon side chains. Nevertheless, it was clear from PCA analysis of lab-scale peated malts and peated new-make spirits that the differentiation due to peat source was similar in both matrices (Figs. 3.17, 3.18, 3.26 and 3.27). To establish the extent of this similarity, the two data sets (malt and spirit) were combined and analysed using PCA. This was possible as many of the same compounds were detected in both malt and new-make spirit.

Ten of the 72 peat-derived compounds detected in new-make spirit were not detected in peated malt (ethylcyclopentanone (**6**), unknown (**8**), unknown (**11**), ethylmethylpyrazine (**12**), diethoxymethylfuran (**14**), methylfuranylpropanone (**30**), unknown (**38**), unknown (**41**), unknown (**46**) and indanone (**55**)). Therefore the remaining 62 compounds detected in both matrices were used as variables for subsequent statistical analysis. To account for the different levels of each compound detected in malt and spirit, the response ratio data for each compound in each matrix was reported relative to the mean response ratio for that compound in that matrix. PCA was then used to detect any similarities in the clustering found in the two data sets (Fig. 3.28).



a



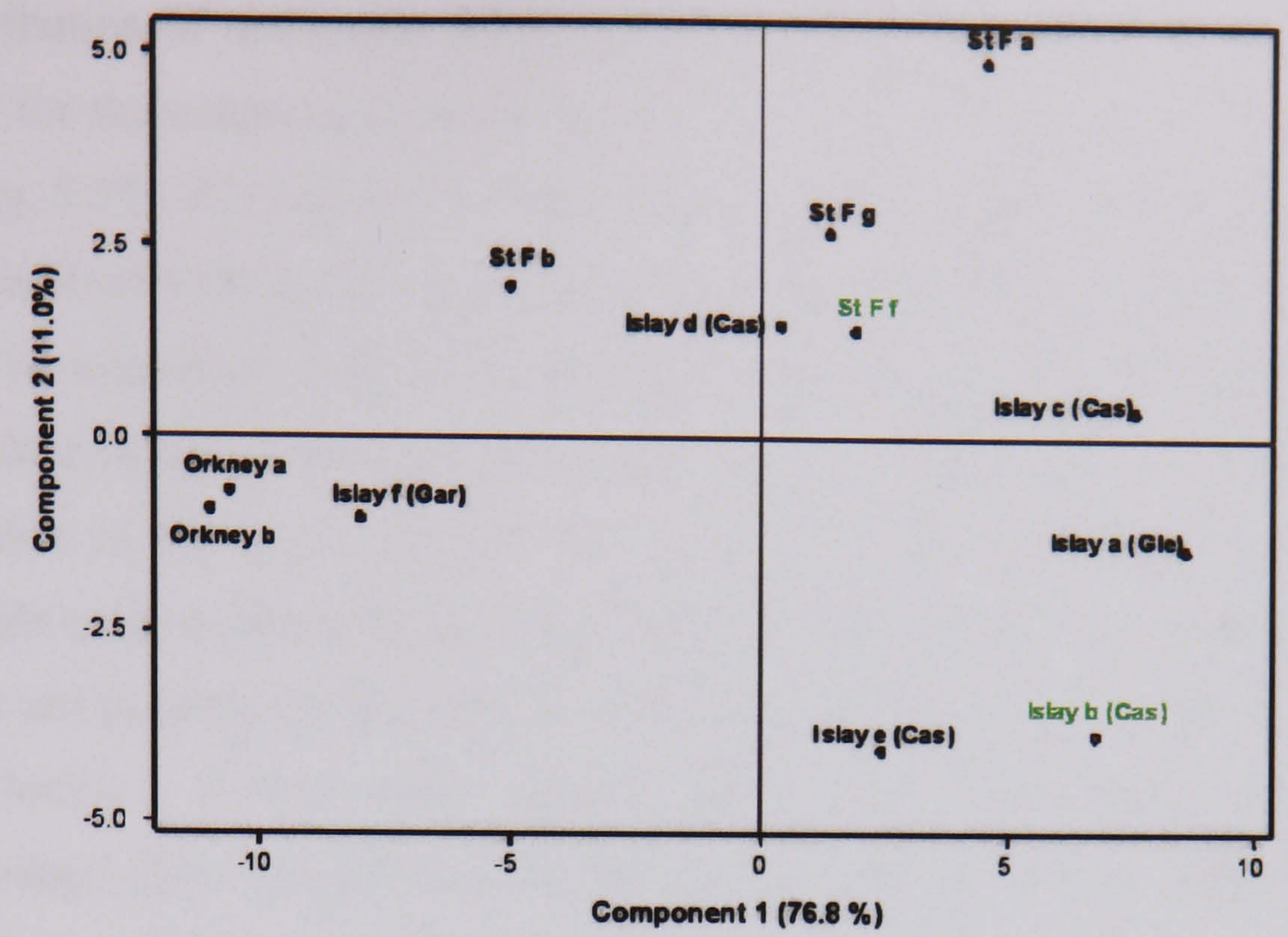
b

Figure 3.28. PCA comparing lab-scale peated malt with lab-scale peated new-make spirit. Sample codes are as described in Table 3.14. The letter m after the sample code indicates malt sample and the letter s indicates spirit. a: Score plot for PCs 1 and 2. b: Score plot for PCs 1 and 3.

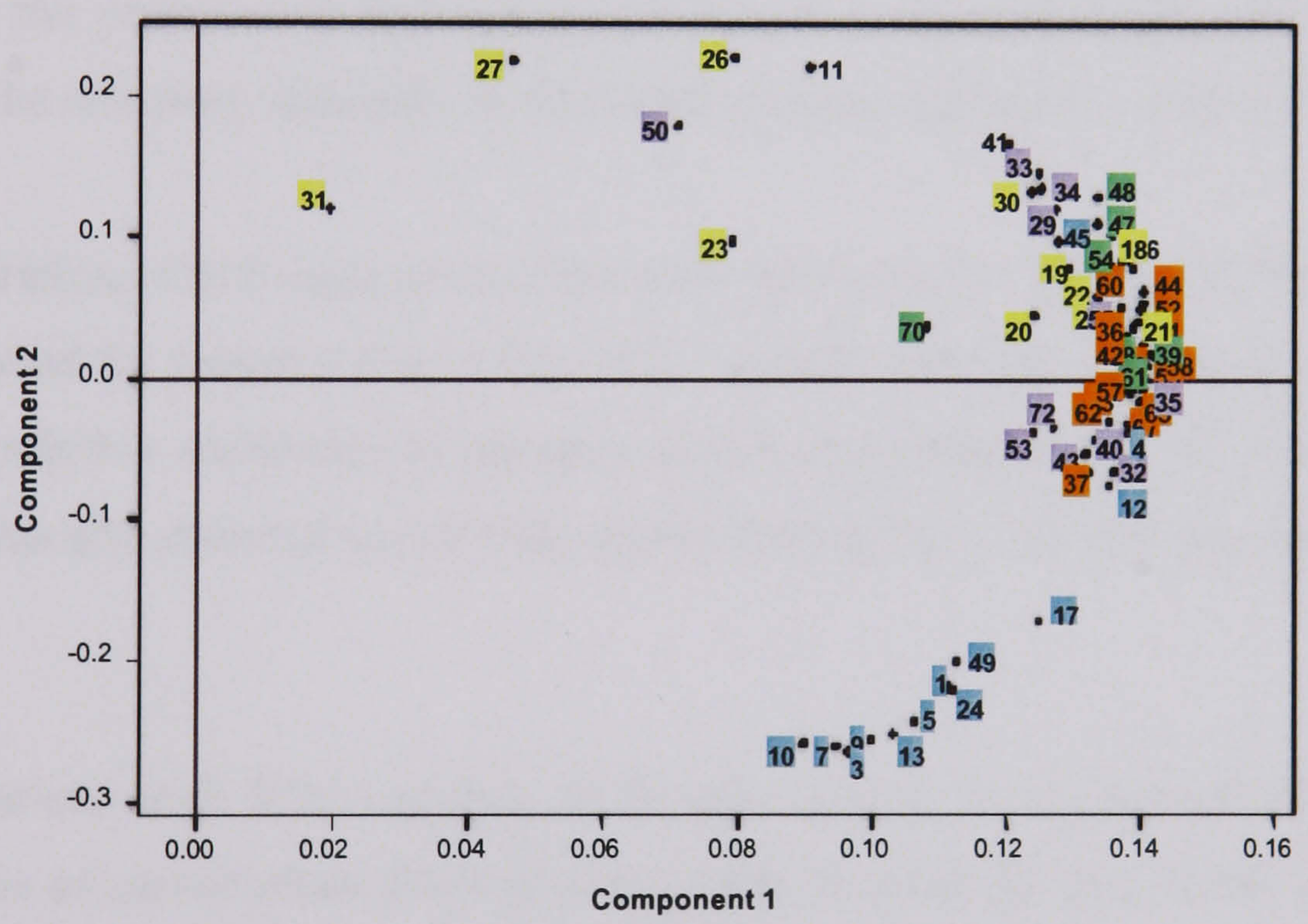
The three PCs shown in Fig. 3.28 show the same separations as were found in both peated malt and new-make spirit. This analysis showed that there was a good correlation in terms of the relative abundances of these compounds in peated malt and peated new-make spirit.

3.3.6 Total chemical profiles of industrially produced peated new-make spirits

Industrial spirit samples produced using five out of the six peat sources used to produce lab-scale new-make spirit were available (no sample manufactured using Tomintoul peat could be obtained). These spirits were analysed using the same GC-MS method that was used for the analysis of the lab-scale spirits (Chapter 2.8). Data for the peat-derived compounds analysed in the industrial spirits are presented in Table 3.19 (Appendix J). Three compounds which were detected in the lab-scale spirits were not detected in the industrial samples: unknown (**8**), benzonitrile (**25**) and unknown (**38**). Therefore, of the 66 compounds found to be significant for the separation of lab-scale spirits in Chapter 3.3.3, 63 were found to be present in the industrial spirits. These compounds were used for PCA of the industrial spirits. As the concentrations and proportions of peat-derived compounds in industrial and lab-scale spirits were different (Tables 3.14 and 3.15 and Fig. 3.22), directly comparing the two data sets was difficult. Therefore, for this analysis, the industrial spirits were analysed independently and the separation of spirits was compared with that found for lab-scale spirits. To relate the separation of spirits in the two data sets, the PCs produced from the industrial data set were explored to identify clustering similar to that found in the lab-scale spirits. The first two components, explaining 87.8% of variance, met this criterion (Fig. 3.29).



a



b

Figure 3.29. PCA of GC-MS data for industrial peated new-make spirits. a: Score plot for PCs 1 and 2, b: Loadings plot for PCs 1 and 2. Compounds are represented by their peak numbers as per Table 3.16 (Appendix H). Colour coding refers to compound classes defined in Table 3.16 (Appendix H).

The distribution of new-make spirits seen using PCA in Fig. 3.29a was similar to that observed for the industrial peated malts in Fig. 3.19a. As was the case with the peated malts (Fig. 3.19), PC 1 shows that the total level of peat-derived compounds played the most significant role in distinguishing between industrial peated new-make spirits (Fig. 3.29). The separation of Hobbister Hill spirit from the Islay and St Fergus spirits on PC 1 may relate to the amount of peat used to produce this spirit as well as to the actual composition of the peat. Indeed, due to the very low peating level, 26 of the 63 compounds used for this analysis were not detected in either of the Hobbister Hill spirits, so it was not possible to characterise these spirits in any way other than their very low peating levels. It was noted though, that a few carbohydrate derivatives – 5-methylfurfural (**23**), 2-acetyl-5-methylfuran (**26**), furfuryl alcohol (**27**) and 5-methyl-2-furanmethanol (**31**) – had lower values for PC 1 indicating that they were, relative to other compounds, more abundant in the Hobbister Hill spirits. This pattern was also found in the peated malt analysis (Fig. 3.19). Lab-scale Hobbister Hill spirit was also found to be relatively abundant in these carbohydrate derivatives (Fig. 3.26).

The separation of St Fergus spirits from Islay spirits on PC 2 in Fig. 3.29 was also similar to that found for peated malts in Fig. 3.19. In both cases the separation was found to be due to a relative abundance of nitrogen-containing compounds in the Islay spirits. This pattern was also detected in lab-scale peated malts (Fig. 3.18) and new-make spirits (Fig. 3.26).

In comparison with Islay samples, St Fergus samples were characterised by a relative abundance of carbohydrate-derived compounds in industrial new-make spirit (Fig. 3.29) as well as in industrial malt (Fig. 3.19). This pattern was, to a lesser extent, detected in lab-scale peated malt (Fig. 3.17) but was not apparent in lab-scale new-make spirit.

3.3.7 Sensory analysis of lab-scale new-make spirits

Peaty aromas

The results of the Quantitative Descriptive Analysis of composite lab-scale new-make spirit samples are shown in Table 3.20. Medicinal was the only attribute not to show significant difference between spirits made using different peat. This was found to be as a result of the low level of variance of the means for the medicinal attribute. Glenmachrie and Castlehill spirits yielded relatively high values for the other peat related flavour attributes (burnt and smoky) as well as for overall intensity of peaty character. St Fergus spirit gave particularly low scores for the intensity of peaty character and burnt aroma. Glenmachrie and Castlehill spirits, along with Gartbreck, gave low values for the presence of other new-make characteristics while St Fergus and, even more so, Tomintoul spirits gave relatively high values for this attribute.

Table 3.20. Aroma scores for lab-scale peated new-make spirits and p values from ANOVA of the new-make spirits where peat source, panellist and session were used as factors.

	Aroma scores				
Sample	**Intensity of peaty character**	**Burnt**	**Smoky**	**Medicinal**	**Other new-make characteristics**
Castlehill	1.7	1.4	1.4	1.0	0.7
Gartbreck	1.5	1.2	1.1	1.0	0.6
Glenmachrie	1.7	1.4	1.4	1.1	0.5
Hobbister Hill	1.5	1.1	1.1	0.9	0.7
St Fergus	1.3	1.0	1.1	1.0	0.8
Tomintoul	1.5	1.2	1.0	1.1	0.9

	p values				
Factor	**Intensity of peaty character**	**Burnt**	**Smoky**	**Medicinal**	**Other new-make characteristics**
Peat source	0.0001	0.0007	0.0000	0.3780	0.0001
Panellist	0.0000	0.0000	0.0000	0.0000	0.0000
Session	0.1049	0.0000	0.4595	0.6801	0.9611

Other new-make characteristics

When carrying out the Quantitative Descriptive Analysis of samples for other new-make characteristics, panellists were asked to try and be specific about what flavours were present. Four commonly identified attributes were sweet, cereal, fruity/ estery and green/ grassy. These attributes were additionally used for Quantitative Descriptive Analysis. ANOVA did not show significant differences for sweet, cereal or fruity/ estery (Table 3.21). There was however, a significant difference for the green/ grassy attribute.

Table 3.21. ANOVA of 6 new-make spirit samples. Peat source, panellist and session were used as factors. Values quoted are p values.

Factor	Sweet	Cereal	Fruity/estery	Green/grassy
Peat source	0.1212	0.2545	0.0595	0.0045
Panellist	0.0000	0.0000	0.0000	0.0000
Session	0.9776	0.8383	0.5282	0.0139

When the values for green/ grassy were plotted, it was found that those samples which yielded the lowest values for other new-make spirit characteristics (the three Islay samples) gave correspondingly low values for this attribute (Fig. 3.30). Hobbister Hill spirit, on the other hand, gave a relatively high value for the green/ grassy aroma.

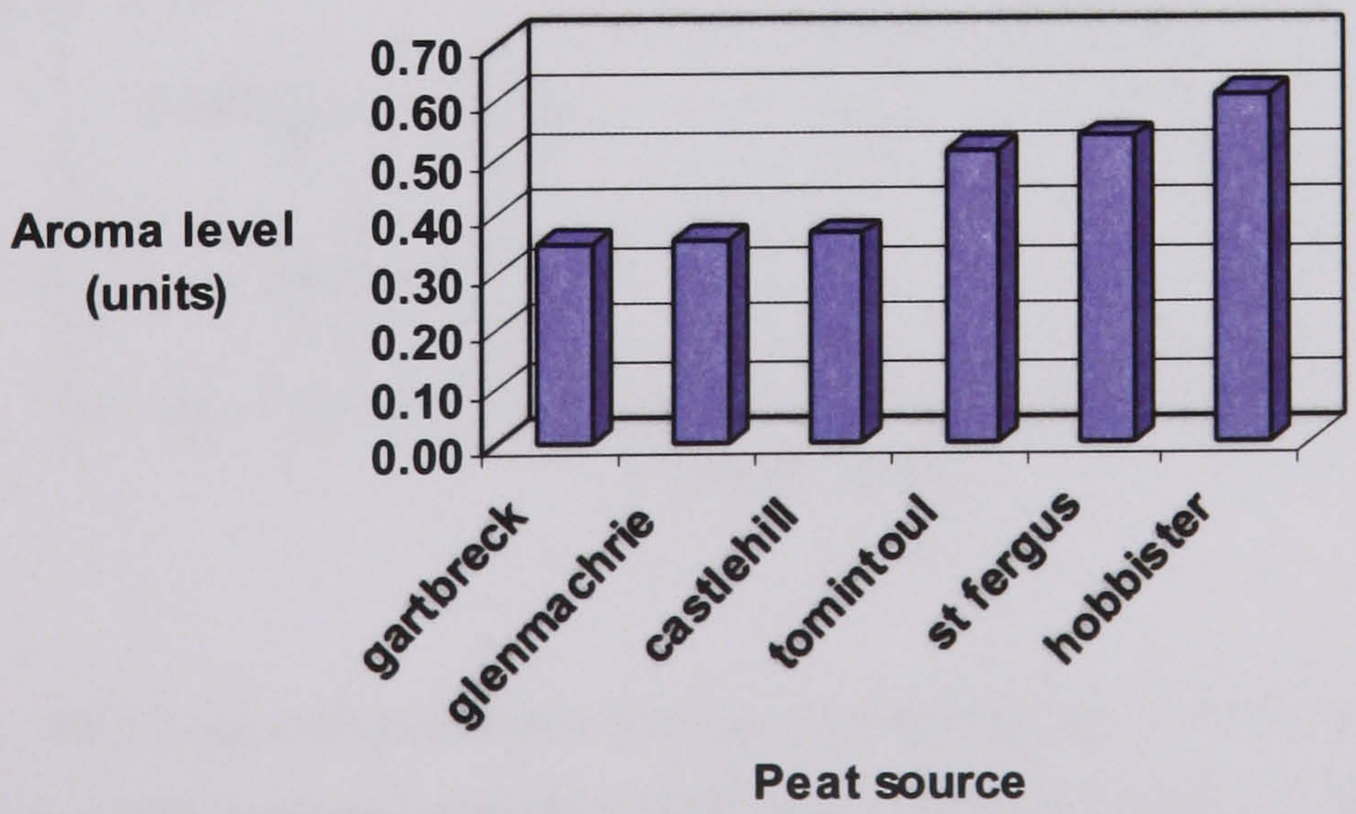


Figure 3.30. Quantitative Descriptive Analysis of green/ grassy aroma. Data are mean values from two sessions.

3.3.8 Comparison of lab-scale and industrial new-make spirit flavour

To see how the flavour of lab-scale peated new-make spirits could be related to industrially produced spirit, it was important to determine how the aromas of the lab-scale spirits compared with those of the spirits produced by industry. Using Quantitative Descriptive Analysis, flavour profiles were produced for the six peated lab-scale spirits and one unpeated lab-scale spirit, with samples being scored for the following descriptors: pungent, sulfury, meaty, solventy, fruity/ estery, green/ grassy, floral, cereal, sweet, soapy, peaty, feinty, oily, sour, stale and clean. These data were compared to flavour profiles previously collected at the Institute for a range of 73 typical new-make malt spirit samples from different distilleries around Scotland. The results of this analysis are shown in Fig. 3.31.

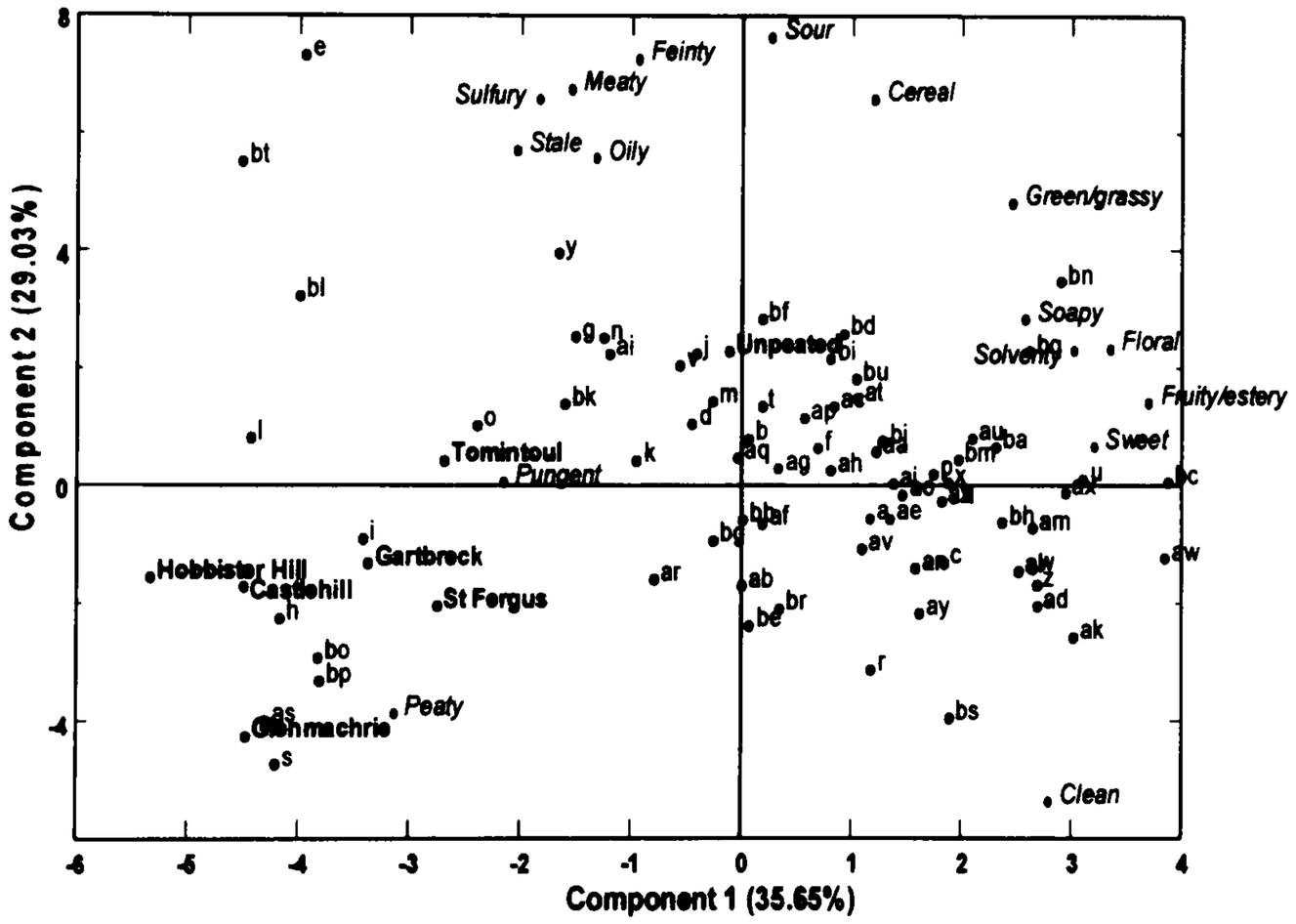


Figure 3.31. PCA bi plot of Quantitative Descriptive Analysis data for lab-scale new-make spirit and industrially produced new-make spirit. Lab-scale peated spirits are labelled using the names of peat source and the lab-scale unpeated spirit is labelled unpeated. The industrial spirits are labelled using letters. PCA loadings (sensory descriptors) are shown in italics.

It was found from the distribution of spirits in Fig. 3.31 that the unpeated lab-scale spirit was similar to the majority of unpeated industrial spirits. In this way, the unpeated lab-scale spirit was not strongly characterised by any sensory terms. The peated new-make spirits were characterised by the peaty term and co-localised well on the PCA plot with industrially produced peated new-make spirits.

Lab-scale and industrially produced peated new-make spirits were compared in more detail. For two peat sources (Castlehill and St Fergus), lab-scale and industrial spirit samples were compared in terms of the three peaty flavour descriptors (burnt, smoky and medicinal) using Quantitative Descriptive Analysis. ANOVA revealed significant differences between lab-scale and industrial spirit with respect to burnt and medicinal aromas (Table 3.22). Comparing the relative intensities of the three attributes, it was found that the industrially produced spirits gave higher values for medicinal aroma, relative to the lab-scale spirits (Fig. 3.32). The reverse trend was found for burnt and smoky which had higher values in the lab-scale samples.

Table 3.22. ANOVA of Quantitative Descriptive Analysis of industrial and lab-scale peated new-make spirits. Sample type = industrial v lab-scale, peat source = St Fergus v Castlehill.

	p values		
Aroma descriptor	**Sample type**	**Peat source**	**Panellist**
Burnt	0.0067	0.2401	0.0008
Smoky	0.0650	0.5610	0.0775
Medicinal	0.0041	0.7835	0.7139

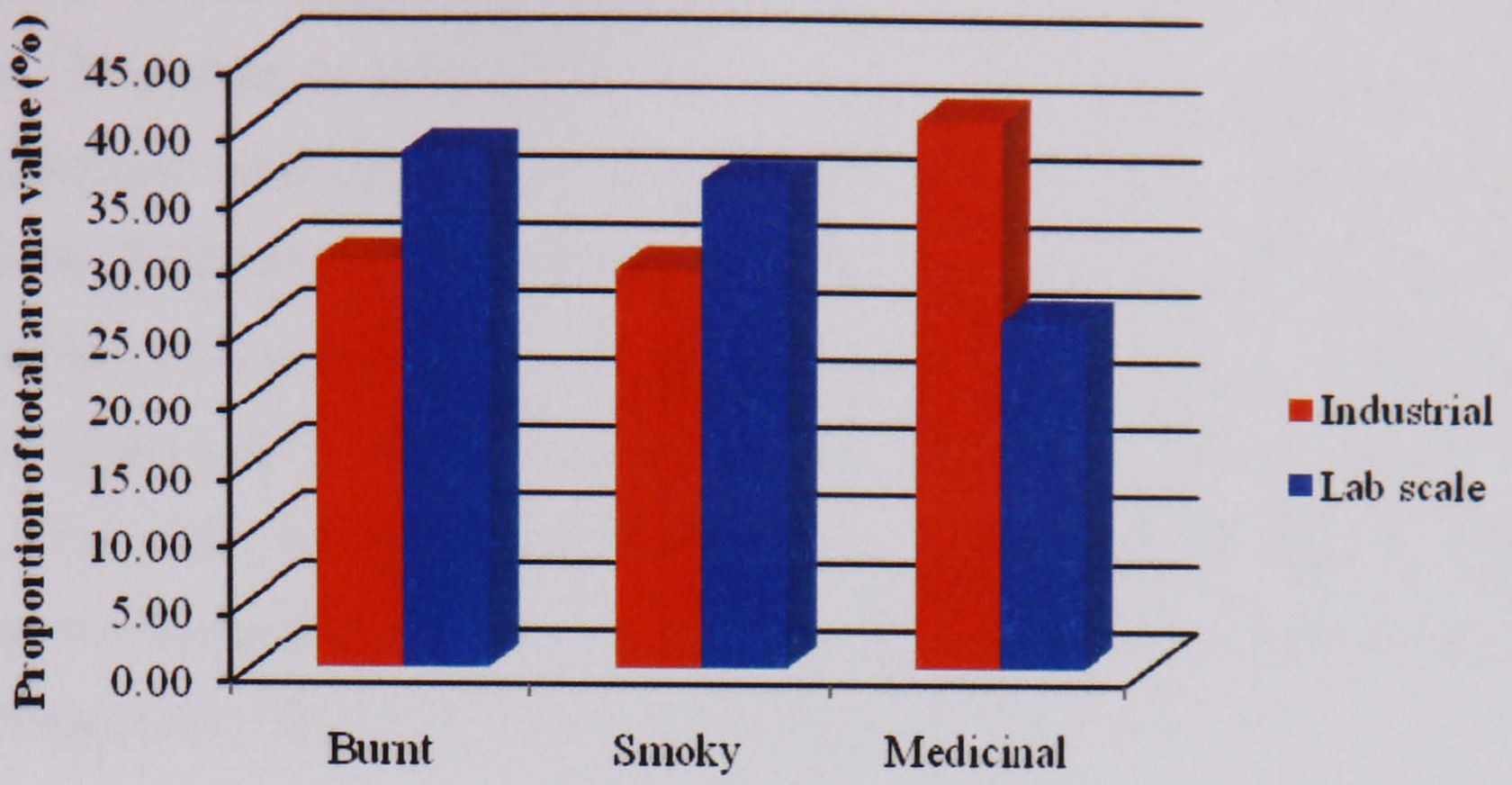


Figure 3.32. Quantitative Descriptive Analysis of lab-scale and industrially produced peated new-make spirits. Values are the aroma intensities for each attribute as a % of the total aroma intensity for each sample.

3.3.9 Identification of organoleptically important peat-derived compounds using GC-O/MS

Aroma detection in lab-scale peated new-make spirit

Composites of three lab-scale new-make spirit samples produced from each of the six peat sources were extracted using SPE and the extracts analysed by GC-O/MS as described in Chapter 2.11. Initially, the aromas detected in these peated new-make spirits were compared with those found in an unpeated lab-scale new-make spirit. In this way, it was possible to determine which aromas were specifically derived from peat and those that were also present in unpeated spirit. Aromas detected in unpeated spirit were removed from subsequent analysis. Aroma descriptions, together with the compound identities (as determined from their mass spectra where detectable) of the 75 peat-derived aromas are shown in Table 3.23 (Appendix K).

The estimated retention indices listed in Table 3.23 were calculated as described in Chapter 2.4.2 with the following adaptation. There was relatively little literature retention index data (which had been obtained using *n*-alkanes as reference substances)

for peat-derived aroma compounds which were identified here by MS with a high degree of certainty. Therefore, to enhance this data set and improve the reliability of the linear equation generated when plotting literature retention index data against experimental retention times, literature retention index data for a group of ethyl esters identified in the new-make spirit extracts by MS were also included. The following ethyl esters were used: ethyl pentanoate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate and ethyl hexadecanoate. Figure 3.33 shows the plot of literature retention index data for compounds identified here with a high degree of certainty by MS versus experimental retention time data.

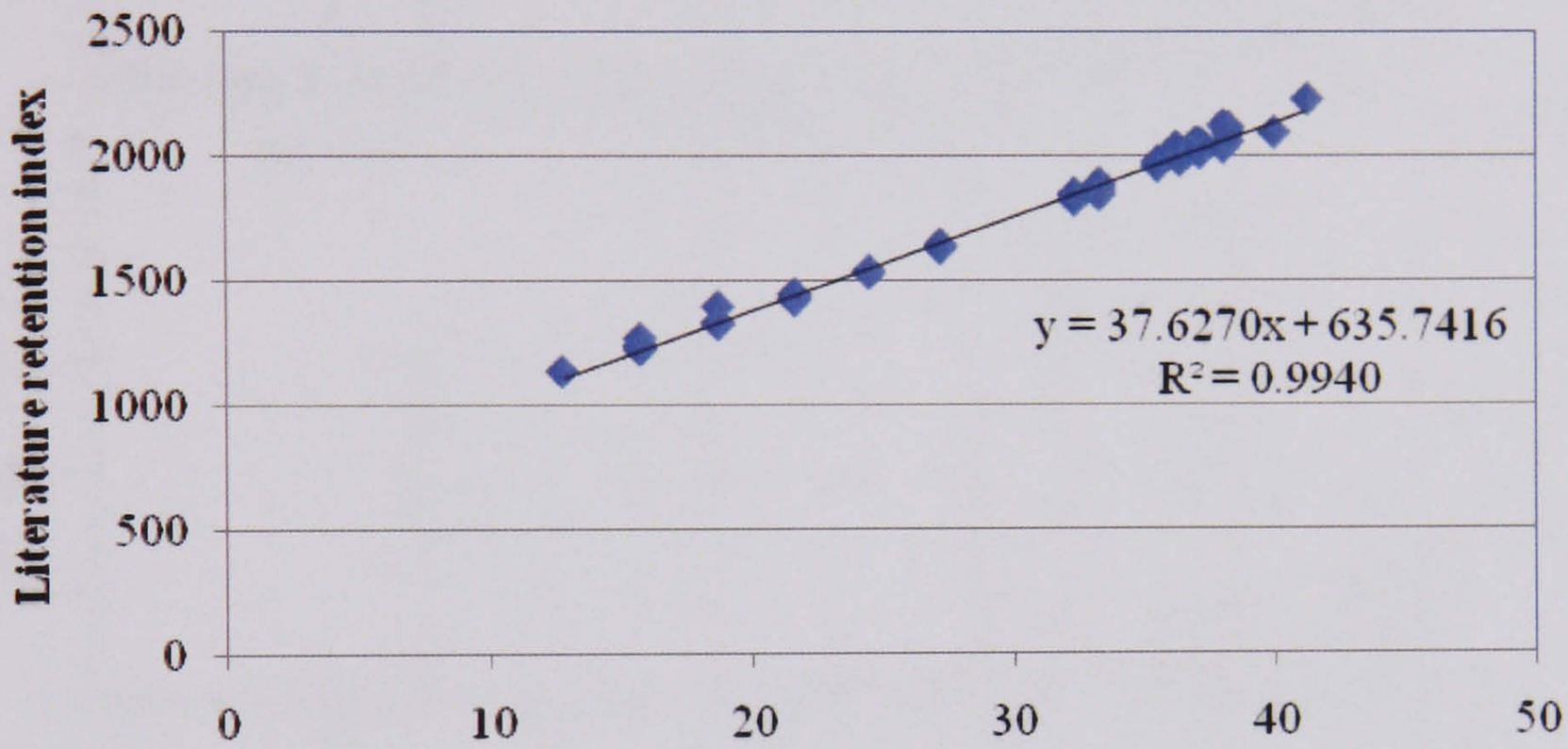


Figure 3.33. Plot of literature retention index data for compounds identified in new-make spirit extracts with a high degree of certainty by MS versus experimental retention time data.

A typical chromatogram obtained from a lab-scale peated new-make spirit is shown in Fig. 3.34 and is compared with one obtained from an industrial sample made using the same peat.

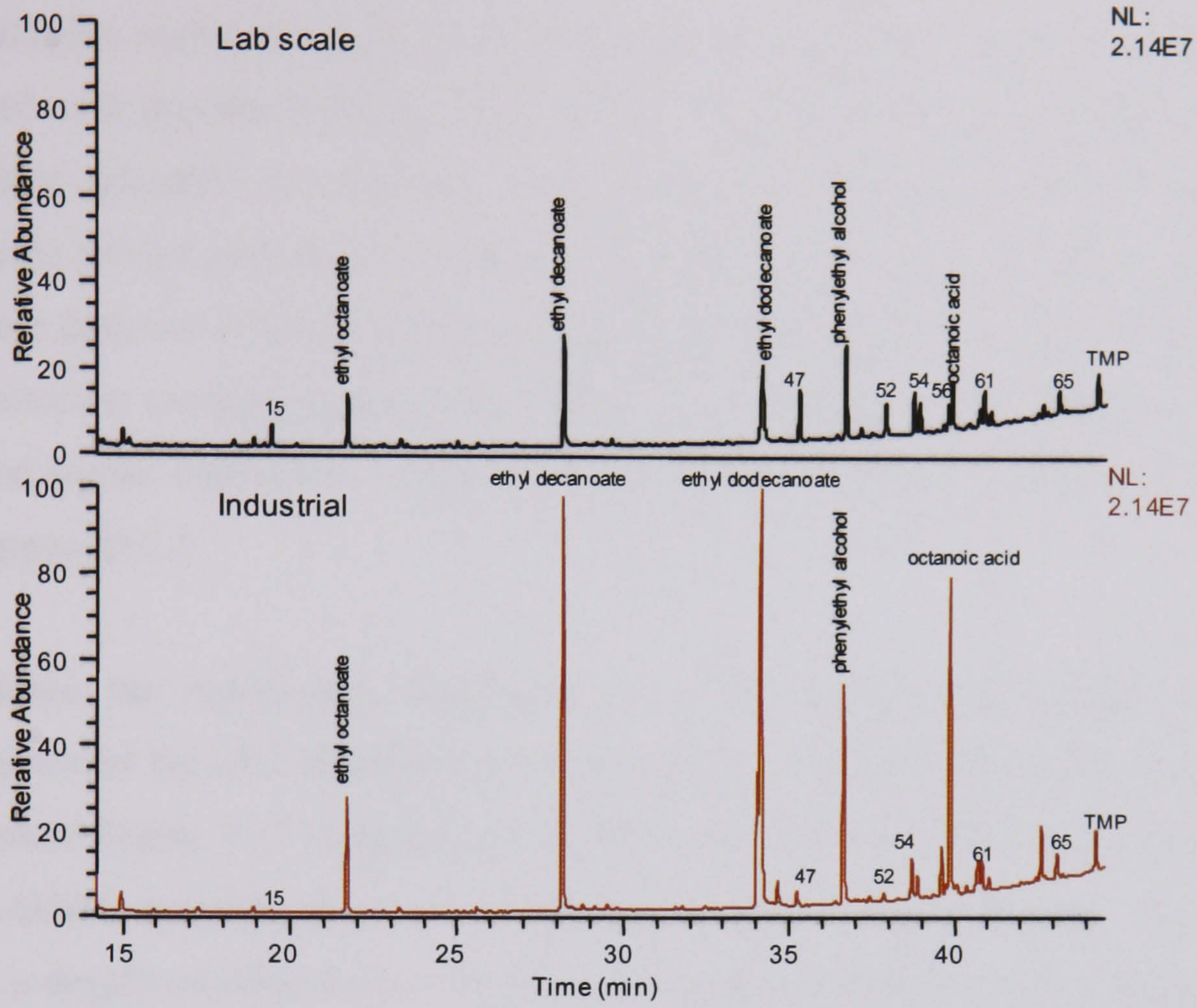


Figure 3.34. Total ion chromatograms obtained from GC-O/MS analysis of solid phase extracts of lab-scale and industrial peated new-make spirits. Both samples were made using the Castlehill peat.

It was apparent from the results shown in Table 3.23 (Appendix K) that there was a difference in the sensitivity of the two assessors carrying out this GC-O/MS work, assessor a detected 68 out of the 75 peat-derived aromas whilst assessor b detected only 33. Interestingly, whilst assessor b tended to detect fewer aromas, seven aromas were detected only by this assessor. The disparity between the two assessors was not equal throughout the GC-O/MS run with the number of aromas being detected being most different during the first half of the run. During the second half of the run, when phenolic compounds were the dominant class, the disparity was less apparent. This result demonstrated the need to use more than one assessor for GC-O/MS analysis.

Forty two peat-derived aromas had an identifiable compound or a clear mass spectrum associated with them. In many cases there was no chromatographic peak present and so identification was impossible. Also, by comparing the area of the peaks found in the unpeated spirit with those found in the peated spirits, it was possible to identify peaks that coincided with peat-derived aromas but were not likely to be responsible for the aroma given their presence in unpeated spirit. Therefore in these cases, the compounds responsible for the peat-derived aromas were unidentified. Some aromas were found to have more than one compound eluting at that time making accurate identification difficult so all potential compounds are listed (Table 3.23 (Appendix K)). The levels of the 46 identified aroma compounds in peated and unpeated lab-scale spirits are listed in Table 3.24 (Appendix L).

To validate the compound identifications of the peat-derived aromas, the aroma descriptions for the 46 compounds were compared, where available, with those found in the literature (Table 3.23 (Appendix K)). Where the specific isomer was not known from the GC-O/MS analysis, data for all reported isomers in the literature were included. Though a degree of subjectivity is involved in assigning descriptors to aromas, there was good agreement, particularly in the case of the phenolic compounds, between the descriptors used during the GC-O/MS analysis and those found in the literature.

Aroma extraction dilution analysis (AEDA)

The GC-O/MS analysis yielded a large number of aromas derived from peat. To determine which compounds were organoleptically most important, AEDA was carried out. Dilutions values used were 10, 100 and 1000 and the results are listed in Table 3.23 (Appendix K). There were 42 aromas with dilution values of 10 or more and the compounds associated with these aromas were identified in 32 cases. All the identifiable phenols had dilution values of 10 or more. Twenty three aromas had dilution values of 100 or more and 17 of these had associated chromatographic peaks which could be identified of which 11 were identified as relating to phenolic compounds. The four aromas with dilution values of 1000 or more were all identified as being derived from

phenolic compounds. It was therefore apparent that phenolic compounds were major contributors to the peaty aroma of the new-make spirits.

Comparison of lab-scale and industrial peated new-make spirits

Once it had been established which compounds were contributing to the aroma of the lab-scale peated new-make spirits, it was important to understand how the composition of this aroma compared to that found in industrially produced new-make spirits. Two industrially produced peated new-make spirits were therefore analysed using GC-O/MS. One spirit had been produced using St Fergus peat (27 ppm total marker phenols) and the other using Castlehill peat (22 ppm total marker phenols).

Twenty four of the 75 peat-derived aromas detected in the lab-scale samples were not detected in the industrial samples (Table 3.25 (Appendix M)). Generally, peak areas (where peaks were detected) were relatively low in the industrial spirits explaining the absence of some aromas, particularly weak aromas, from these samples. Indeed, 18 of the 24 aromas not identified in industrial spirit had dilution values of only 1. Only six out of 42 aromas with dilution values of 10 or more were not detected in the industrial samples (3-ethylcyclopentanone (**14**), unknown (**19**), ethyl 7-octenoate (**20**), unknown (**55**), 3-ethylphenol (**66**) and unknown (**69**)). It must be noted that unknown (**19**) was found in industrial samples at 28.74% of the level found in lab-scale samples and the dilution value for this aroma was 100. Therefore an aroma might be expected for this compound in the industrial spirits so this may not be the aroma active compound. The lack of an aroma for 3-ethylphenol in the industrial samples could be due to a masking effect of the 4-ethylphenol aroma.

As the dilution values are increased further, the proportion of aromas undetected in the industrial samples dropped further. Only two out of 23 aromas with dilution values of 100 or more were undetected in the industrial samples (unknown (**19**) and unknown (**55**)) and all four aromas with a dilution value of 1000 or more were detected in the industrial samples. Also, all aromas assigned to phenolic compounds, with the possible exception of 3-ethylphenol, were detected in the industrial spirits. This was important as these

compounds were, as stated above, found to be the most potent group of odorants contributing to the peaty aroma.

Ethyl 5-heptenoate (**16**) was detected in the lab-scale samples but was not detectable in the industrial samples. An aroma was detected when this compound eluted in both lab-scale and industrial samples. It seems unlikely, therefore, that this was the compound contributing to the aroma. Similarly for 1-phenyl-2-propanone (**38**), an aroma was detected in both lab-scale and industrial samples but, given the low levels detected in the industrial samples, this compound would not be expected to be aroma active and so again this may not be the compound contributing to the detected aroma.

3.3.10 Multivariate statistical analysis of combined analytical and sensory data from new-make spirits produced using peats from different locations

The lab-scale peated new-make spirits were compared using a combination of analytical data and sensory data in order to ascertain which compounds were most likely to be contributing to peat-derived aromas. The analytical data were the GC-MS peak area data for the compounds identified using GC-O/MS as contributing to the peaty aroma (Table 3.24 (Appendix L)). These data could be used to indicate which peat-derived aroma active compounds identified by GC-O/MS were characteristic of particular new-make spirit samples. These analytical data were then combined with the sensory data obtained using Quantitative Descriptive Analysis in Chapter 3.3.6 and the two data sets were analysed together by PCA. In this way it was possible to determine how individual compound aromas related to the overall peaty aroma of the spirit.

The first three PCs produced by PCA respectively explained 43.63%, 29.12% and 22.71% of the total variance (a total of 95.47%). These three PCs were therefore plotted. On the score plot of PCs 1 and 2 (Fig. 3.35a), it can be seen that Glenmachrie, Castlehill and St Fergus spirits had high positive values on PC1 whilst Tomintoul and Hobbister Hill spirits had negative values with Gartbreck located close to the origin. PC 2, on the other hand, separated St Fergus spirit from Castlehill and even more so from

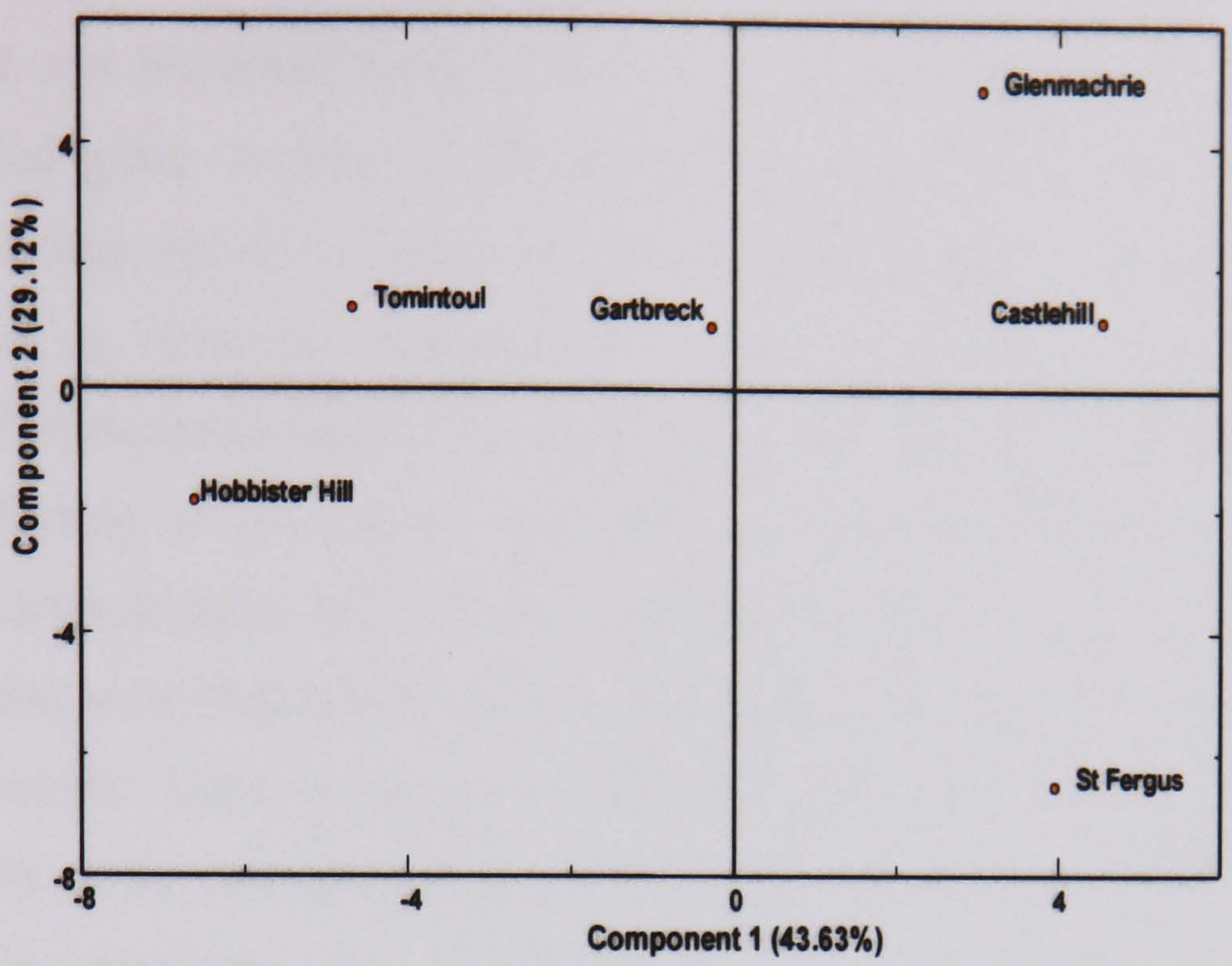
Glenmachrie. Relating this separation to the sensory descriptions given by Quantitative Descriptive Analysis, the three peaty descriptors (burnt, medicinal and smoky) all co-localised along with the overall intensity of peaty character to the upper right quadrant of the loadings plot (Fig. 3.35b). This reflected the fact that all of these aromas were most intense in the Castlehill and Glenmachrie spirits. Given this information, it would be expected that aroma compounds which located to the upper right quadrant of the loadings plot would give the greatest contribution to the peaty aromas. As a group, the phenols were found to be the most prominent compounds in the upper right quadrant of the plot (Fig. 3.35b). Some phenols were found to be exceptions to this finding: 2-chlorophenol (**45**) and hydroxymethylacetophenone (**50**) located to the upper left quadrant and *m*-cresol (**61b**) and 3-ethylphenol (**66**) located to the lower right quadrant. The aroma of the phenols, as described by GC-O/MS analysis, was generally described as medicinal. This result, combined with the fact that these compounds had dilution values of 10 or more (Table 3.23 (Appendix K)), suggests that these compounds are the major contributors to the peaty aroma of the spirit.

Also locating to the upper right quadrant were three guaiacyl compounds: guaiacol (**47**), methylguaiacol (**52**) and ethylguaiacol (**56**). This group of compounds had aroma descriptors which were quite distinct from the phenols and were described as smoky, sweet and spicy. From the dilution analysis, it was apparent that guaiacol was a particularly potent contributor to the peaty aroma with a dilution value of 1000. Although methylguaiacol and ethylguaiacol did not have such high dilution values (10 and 100 respectively) they are both likely to contribute to spirit aroma.

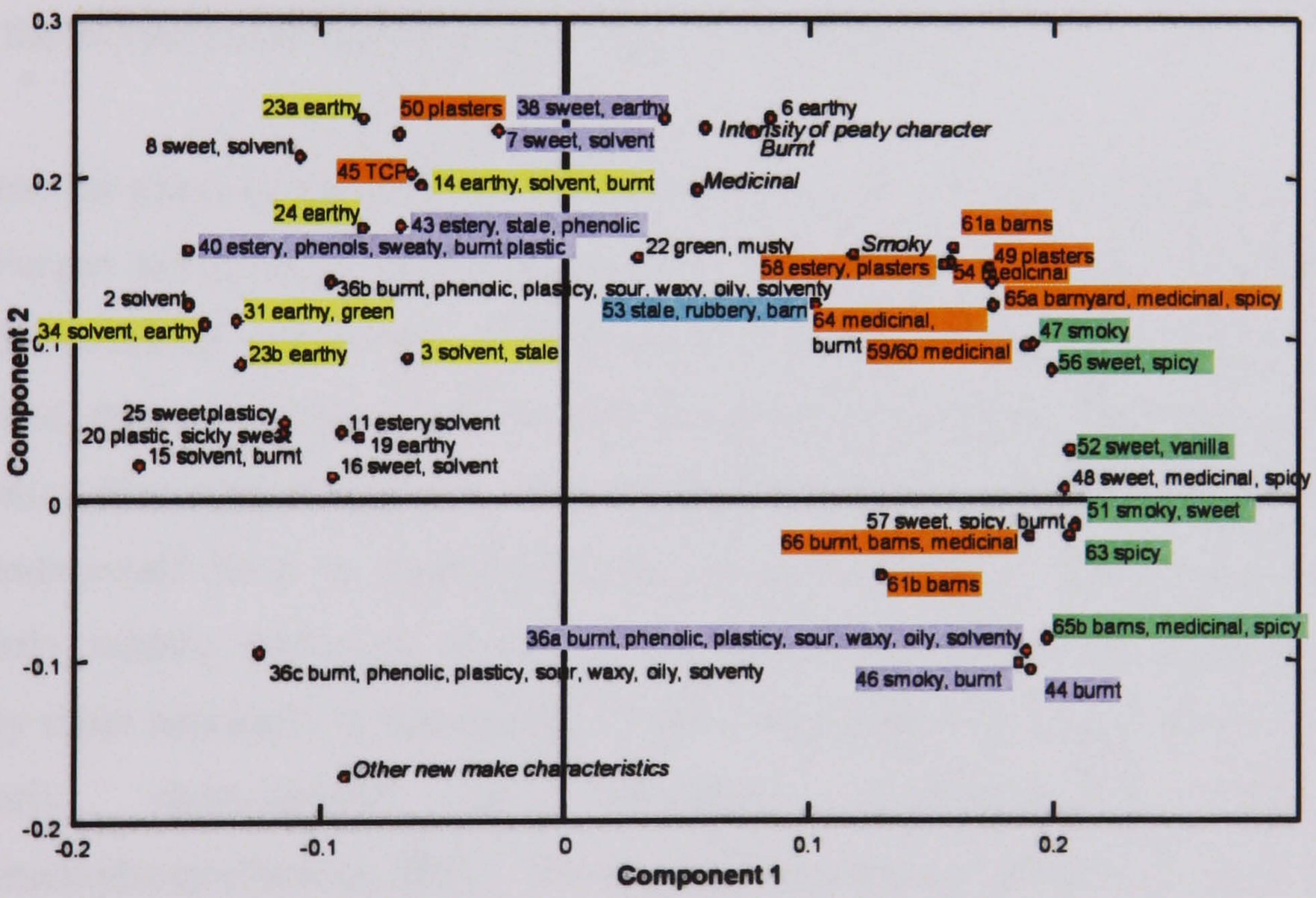
A few additional compounds also located to the upper right quadrant of the loadings plot. Quinoline (**53**), which was the only identified nitrogen-containing compound, had an aroma described variously as stale, rubbery and barn-like. This aroma had a dilution value of 100 so is likely to contribute to the spirit aroma. One aromatic compound, 1-phenyl-2-propanone (**38**) and one unknown (**48**), which given its mass spectrum is likely to be aromatic in nature, located to the upper right quadrant. However, with dilution values of only 1, these compounds probably did not contribute significantly to spirit

aroma. The same could be said for the alcohol, 6-hepten-1-ol (**22**). The other compound located in the upper right quadrant of the plot was ethyl *trans*-2-pentenoate (**6**) which was described as having an earthy aroma with a dilution value of 100. So this compound may contribute to the spirit aroma though not necessarily to the specific peaty aromas analysed here.

PC 2 described a relatively large percentage of variance (29.12%) and so the separation of St Fergus spirit from the Islay spirits on this component must be considered of importance. The St Fergus spirit was separated from the Islay spirits on PC 2 due to a particular abundance of certain phenols (*m*-cresol (**61b**) and 3-ethylphenol (**66**)), guaiacols (methoxymethylphenol (**51**), propylguaiacol (**63**) and eugenol (**65b**)) and aromatic compounds (dimethoxybenzene (**36a**), dimethoxytoluene 1 (**44**), dimethoxytoluene 2 (**46**) and unknown (mass spectrum suggests aromatic compound) (**57**)) (Fig. 3.35b). With the exceptions of dimethoxybenzene (**36a**) and dimethoxytoluene 1 (**44**), these compounds all had dilution values of 10 or more. The aromas of these compounds were largely described as smoky, spicy and burnt. It was surprising therefore, to find that the relative abundance of these compounds in the St Fergus spirit should confer relatively low aroma intensities for the peaty descriptors in this spirit. Furthermore, this particular spectrum of compounds in the St Fergus spirit also resulted in a relatively high intensity of 'other new-make characteristics'. However, the sensory analysis carried out in Chapter 3.3.6 was unable to identify any particular other new-make characteristics which characterised the St Fergus spirit.



a

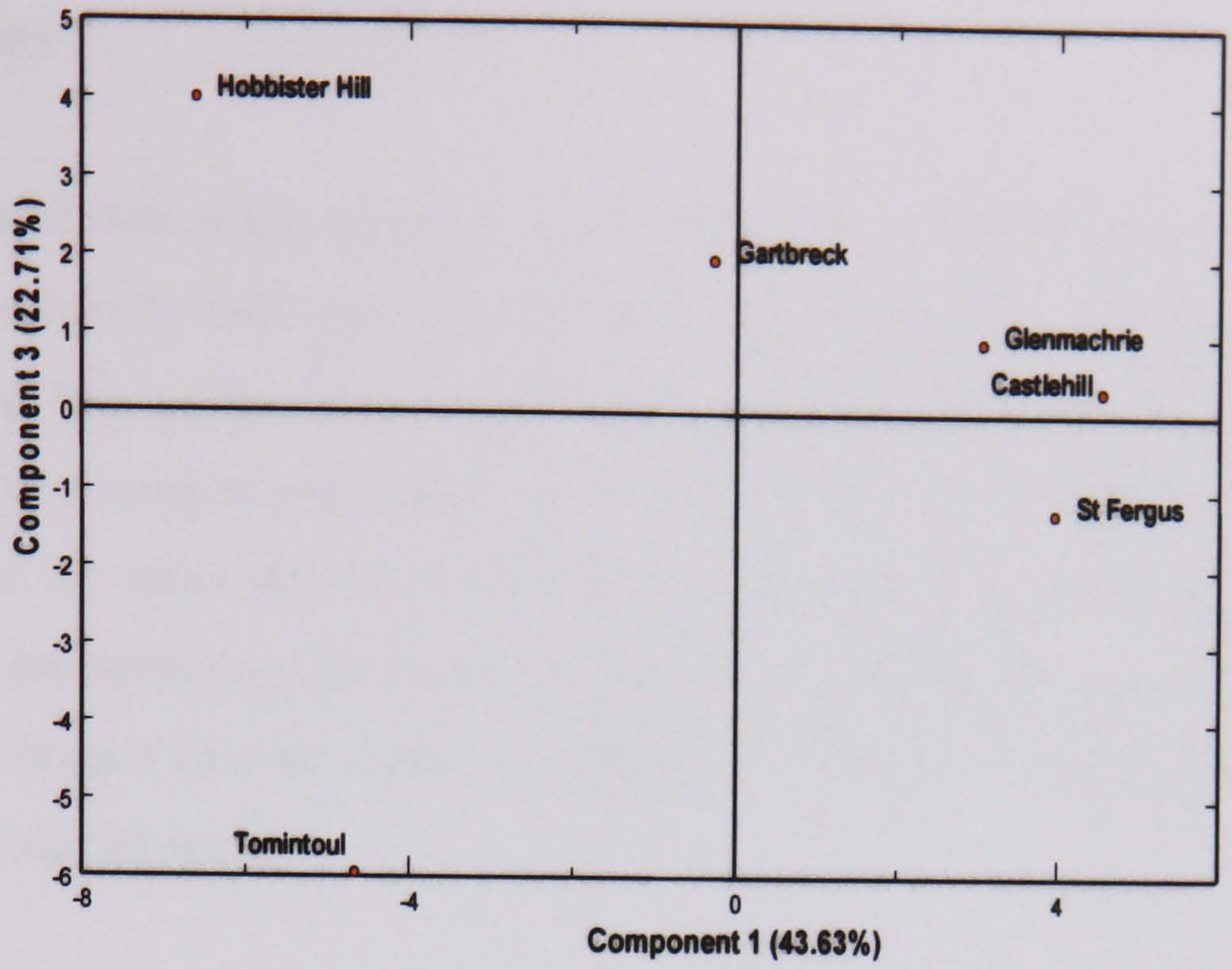


b

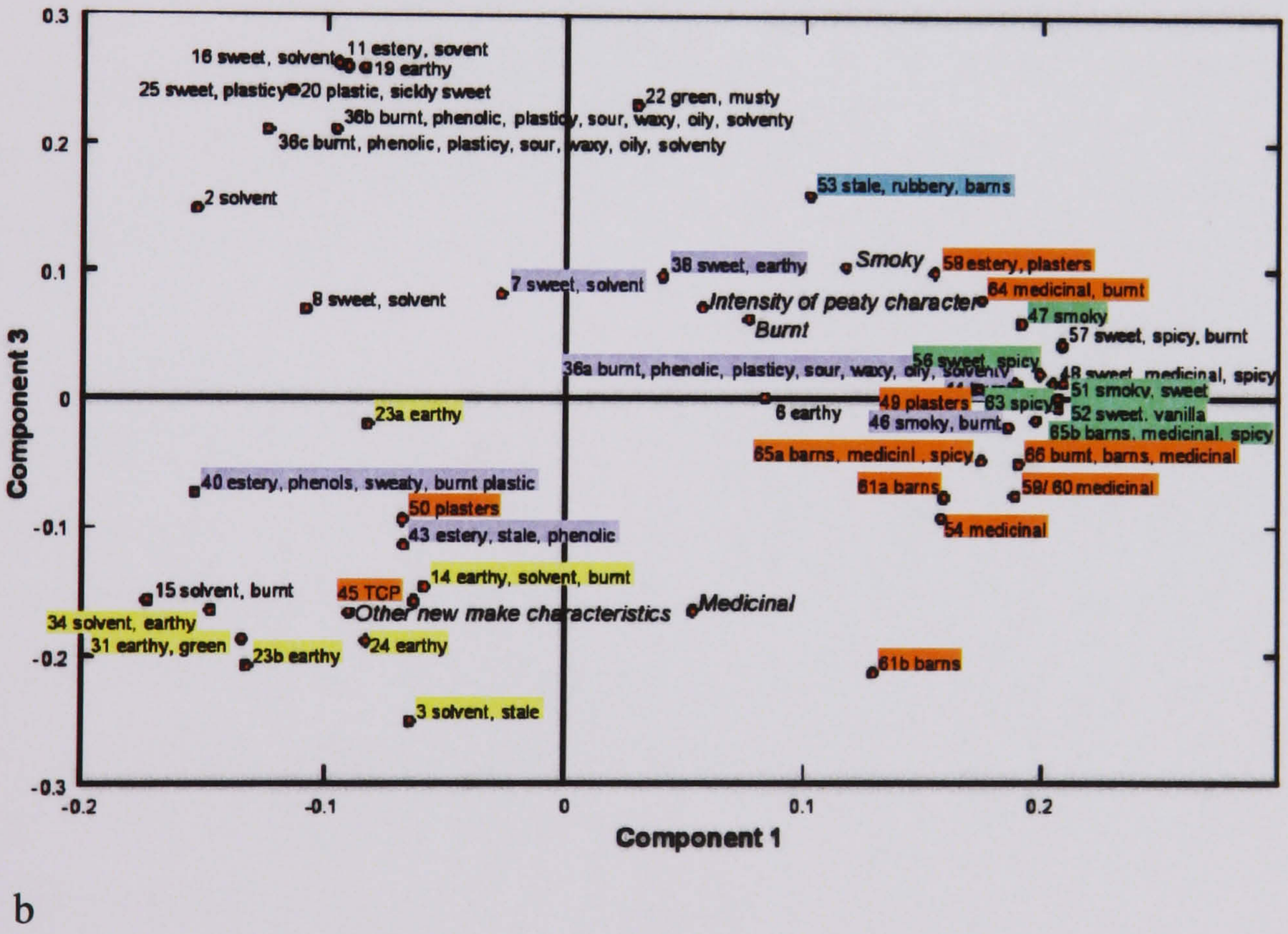
Figure 3.35. PCA of combined sensory and analytical data. a: Score plot for PCs 1 and 2. b: Loadings plot for PCs 1 and 2. Quantitative Descriptive Analysis descriptors are shown in italics. Compounds are represented by their peak numbers and odour descriptions as per Table 3.23 (Appendix K). Colour coding refers to compound classes defined in Table 3.24 (Appendix L).

As shown in Fig. 3.36, PC 3 also explained a relatively large percentage of variance (22.71%) and was important predominantly for the separation of Hobbister Hill spirit from Tomintoul spirit. In terms of Quantitative Descriptive Analysis aroma descriptors, this separation was due to a relatively intense medicinal aroma in the Tomintoul spirit. The difference in chemical composition in the two spirits was due largely to the abundance of carbohydrate-derived compounds in the Tomintoul sample and a relative abundance of many of the 'other compounds' in the Hobbister Hill spirit. The 'other compounds' characterising the Hobbister Hill spirit were mostly made up of ethyl esters and their aromas were described as sweet and solventy. Neither the carbohydrate-derived compounds nor the 'other compounds' characterising Hobbister Hill had aromas which related directly to the peat-derived aroma descriptors from the Quantitative Descriptive Analysis. In particular, the carbohydrate-derived compounds characterising the Tomintoul spirit generally had earthy aromas and so these compounds did not appear to explain the elevated medicinal aroma in the Tomintoul spirit.

In general, the phenols, guaiacols and aromatic compounds which characterised the Islay and St Fergus spirits had a relatively small influence on the separation of the Hobbister Hill and Tomintoul spirits (Fig. 3.36b). However, as compounds belonging to these classes had aromas which correlated well with the peaty sensory descriptors from the Quantitative Descriptive Analysis, any distinction between spirits in terms of these compounds could have an important effect on spirit aroma. With reference to the particularly intense medicinal aroma in the Tomintoul spirit, several phenols were relatively more abundant in this sample. Two compounds belonging to this class were particularly characteristic of Tomintoul: 2-chlorophenol (**45**) and hydroxymethylacetophenone (**50**). These two compounds, along with several other phenols were described as having medicinal type aromas and so could contribute to the elevated level of medicinal aroma in Tomintoul spirit.



a



b

Figure 3.36. PCA of combined sensory and analytical data. a: Score plot for PCs 1 and 3. b: Loadings plot for PCs 1 and 3. Quantitative Descriptive Analysis descriptors are shown in italics. Compounds are represented by their peak numbers and odour descriptions as per Table 3.23 (Appendix K). Colour coding refers to compound classes defined in Table 3.24 (Appendix L).

3.3.11 Summary

Producing peated new-make spirit on a lab-scale, it was possible to see that some peat-derived compounds do not survive the mashing, fermentation and distillation processes. Notable among this group were the lignin-derived syringols. Nevertheless, analysis of the levels of the remaining compounds showed that peat source had a direct impact on the composition of the spirit and the variation in the levels of peat-derived compounds was found to have an impact on the flavour of the spirit. Despite the variation in production processes, the impact of peat source on chemical composition could also be detected in industrially produced spirits.

Chapter 4: Discussion

4.1 Peat

The ultimate goal of this project was to determine whether the origins of peat used for malt kilning had an impact on the flavour of peated new-make spirit. To answer this question, the first objective was to analyse peat from different locations to determine whether the location had an impact on peat composition. Peat samples from different locations were successfully differentiated using FT-IR. Subsequent Py-GC-MS analysis showed which chemical compounds were responsible for this differentiation.

4.1.1 Peat differentiation due to geographical location

To determine whether peat sourced from different locations around Scotland could be chemically differentiated, FT-IR was used as a high-throughput technique to screen a large number of peat samples.

From the results of the FT-IR analysis, the separation of Tomintoul and St Fergus peats from each other as well as from the rest of the peat sources was notable as these deposits were the two peat sources on the mainland of Scotland, the two sites being situated in two distinct areas in the north east of Scotland. The chemical differentiation between peat samples from the two mainland sources may reflect different vegetational inputs to the peat at each location. Tomintoul and St Fergus are both considered basin bogs and the type of vegetation found in these bogs can be relatively heterogeneous and varies according to ground water input [35]. In this way, Tomintoul peat contained a high percentage of moss and the St Fergus peat contained a high percentage of woody material.

One reason for the distinction between island and mainland peat could be the different conditions under which they were formed. The relatively high rainfall to which the island

peat is exposed to [121] may increase oxygen flow into the upper regions of the peat thus enhancing aerobic decomposition. Additionally, the island peat deposits were formed in regions exposed to high winds [121]. These high winds may aerate and, to an extent, desiccate the upper regions of peat again encouraging aerobic decomposition. Taking these factors together, it is proposed that island peat is relatively decomposed when compared with mainland peat. It was hard to establish any obvious general differences in physical appearance between island and mainland peats that could confirm this speculation.

The relative similarity of Islay and Hobbister Hill peat may reflect the fact that in the relatively acid, low nutrient basin bogs of the west and north of Scotland the predominant plant species are varieties of *Sphagnum* as well as such plants as sedges, heather and cotton grass which are species also common to blanket bogs [35]. Therefore, the vegetation contributing to peat at the island locations, irrespective of deposit type, is similar. What difference there was between Hobbister Hill and Islay peat may be due, at least in part, to the relative abundance of *Calluna vulgaris* in peat deposits in the north of Scotland compared with the south west [122]. This particular species tends to accumulate in relatively dry peat deposits suggesting that Hobbister Hill peat is drier than Islay peat [64]. An increased prominence of a particular plant species such as this may have an effect on the chemical composition of the peat. Furthermore, as the elevated abundance of *Calluna vulgaris* suggests that Hobbister Hill peat is relatively dry, this may mean that peat at this site is more susceptible to decomposition.

Small though it was, the differentiation of Islay peat, namely Gartbreck and Glenmachrie from Castlehill, may be due to a slightly higher degree of decomposition of the Castlehill peat [123]. This elevated decomposition in the Castlehill peat may be due to the sloping nature of the site allowing increased drainage to occur.

4.1.2 Peat characterisation due to geographical location

Using FT-IR it was possible to distinguish between peat samples according to their geographical source. However, as FT-IR provided no information concerning the identity of the compounds responsible for this differentiation, another method was utilised for this purpose. Peat samples from each of the differentiated locations identified using FT-IR were therefore analysed using Py-GC-MS. This analysis was able to define the compounds responsible for the differentiation of peats.

Phenolic compounds

Phenolic compounds (including guaiacols, syringols and phenols) are known to be important for smoke flavour, therefore the observed differences in the relative concentration of phenolic compounds to be found in peats from different sources are of obvious interest to the maltster [85]. The fact that the proportion of phenolic compounds differed significantly between peat sources indicated that, when used for kilning, some peats may yield higher concentrations of these important smoke flavour compounds than others.

Peat samples from Tomintoul contained significantly less lignin derivatives than the St Fergus peat samples despite being taken from a similar deposit type in a similar area of Scotland. This chemical difference could be related to the presence of a relatively high proportion of woody material in the St Fergus samples yielding high amounts of lignin derivatives while the *Sphagnum* moss, evident in the Tomintoul samples, lack lignin [46]. The amount of extraction that has taken place at St Fergus and Tomintoul may have had an impact on the vegetation present in the peat. The upper horizon of raised bogs, which has risen above the water table, tends to be characterised by *Sphagnum* mosses [35]. This may be the horizon from which Tomintoul peat is currently being extracted and would give a reason for the low lignin level in Tomintoul peat. Below this upper horizon, where peat has formed under the influence of ground water input from surrounding soil, vegetation consists of a higher vascular plant content [35]. St Fergus peat samples may

therefore represent older peat which has been exposed by extensive peat extraction at this site.

There were several guaiacyl-derived carbonyl compounds which were more abundant in the island samples relative to other lignin derivatives. Most notable in this respect was the relative increase in abundance of acetovanillone in the island peat. This compound has previously been identified as one which reflects an increase in lignin degradation, probably due to fungal action which causes an increase in the relative abundance of lignin compounds containing a carbonyl group [47]. This finding may therefore suggest an increase in fungal activity in the island peat deposits. Also, it has been shown previously that syringyl compounds are degraded preferentially over guaiacyl compounds during peatification [45]. The high percentage of syringyl compounds in St Fergus peat may indicate that it has undergone less lignin decomposition than peat from the other deposits. However, the syringyl to guaiacyl ratio also depends on the species of source vegetation and so without prior knowledge of the contributing species it is not possible to determine whether the relatively high percentage of syringyl compounds in the St Fergus peat is due to a lack of decomposition alone [51].

The Islay peat samples were relatively rich in ferulic acid and its pyrolysis product vinylguaiacol [124]. Grass cell walls contain high concentrations of ester-linked *p*-coumaric and ferulic acids, while woody and legume plants have little of these constituents [125,126]. This finding suggests a relatively high input of grassy type material to the Islay peat samples.

Some non-methoxylated phenolic compounds were found to be characteristic of St Fergus peat. Among these, the propenyl phenols may be lignin pyrolysis products [46]. 3-Methoxy-1,2-benzenediol may represent an intermediate in the breakdown of syringyl lignin as demethylation of methoxy groups is known to be a common microbially induced modification [127]. Methylbenzenediol and 4-ethylcatechol may be derived from tannins [64].

One phenolic compound, 2-ethylphenol, was particularly characteristic of Hobbister Hill peat. In a study carried out on *Sphagnum* leaves, the rootlets of *Ericaceae* (heathers) and their associated peat, the only fraction to contain this compound was the peat [46]. This suggests that this particular compound accumulates in biodegraded material though it is not certain what the parent structure may have been. The inference is that Hobbister Hill peat is particularly biodegraded.

Carbohydrate derivatives

Samples from Tomintoul were found to contain high levels of moss remains and were all high in carbohydrate derivatives. Increased levels of furans, pyranones, cyclopentanones and cyclopentenones derived from plant carbohydrates have been shown to indicate *Sphagnum* moss in peat as *Sphagnum* remains consist almost entirely of non-biodegraded polysaccharides [46]. Therefore the relative abundance of these carbohydrate derivatives in Tomintoul peat may reflect the observed abundance of moss remains in these samples.

4-hydroxy-5,6-dihydro-(2H)-pyran-2-one, considered a xylan marker [128], was, relative to the other carbohydrate derivatives, more abundant in St Fergus and Tomintoul peat than Islay and Orkney peat. Hemicellulose-derived pentosans, such as xylan, have been found to be preferentially degraded over cellulose and other hexose sugars [45]. The implication was, therefore, that Islay and Orkney peats were more degraded than those from St Fergus and Tomintoul.

Whilst 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one was relatively most abundant in St Fergus and Tomintoul peats, there was an additional source of variation where this compound co-localised with a group of compounds which together characterised Islay peats. This group of compounds included ferulic acid which, as has previously been suggested, may accumulate in peat containing an abundance of grass type vegetation. In grasses, *p*-coumaric and ferulic acids are known to be linked, through arabinose, to xylans [129]. It may be that xylan, and therefore 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one, is also particularly characteristic of the grassy vegetation typical of these samples.

Another carbohydrate derivative, 2-methyl-5-hydroxy-(4H)-pyran-4-one, on the other hand, was proportionally more abundant in Islay and Orkney peat. In a study carried out on *Sphagnum* leaves, the rootlets of *Ericaceae* and their associated peat, the only fraction to contain this compound was the peat suggesting that it is a product of biodegradation [46].

Nitrogen-containing compounds

Several nitrogen-containing compounds- benzyl nitrile, indole, 3-methyl-1H-indole, pyridine, methylpyridines, 2-pyridinecarbonitrile, pyrrole and 1-methyl-1H-pyrrole - were found in the peat samples. It has been shown previously that nitrogen-containing compounds tend to accumulate in more degraded peat [130]. One potential source of nitrogen-containing compounds in peat are the lignin degrading fungi where pyridine and its derivatives could be derived from the chitinous components found in these organisms [131] and pyrroles, indoles and benzyl nitriles could be derived from proteinacious material [132,133]. Relative to the amount of lignin derivatives in the peat, these compounds were generally found to be characteristic of Islay and Hobbister Hill peat. Therefore, the presence of an elevated abundance of nitrogen-containing compounds in these samples supports the hypothesis that these samples are relatively biodegraded.

Aromatics

Several aromatic compounds were found to be characteristic of the Orkney samples. These compounds may represent the products of lignin or other polyphenolic structure degradation [45]. Reportedly, facultatively anaerobic bacteria, for example *Enterobacter*, *Escherichia* species, can transform lignin degradation products (e.g. ferulic acid) under anaerobic conditions into toluene, ethylbenzene as well as phenols [134]. More specifically, styrene may have been generated by *O*-demethylation, dehydroxylation and decarboxylation of ferulic acid before further reduction of the double bond in the side-chain to ethylbenzene [135].

Another aromatic compound of interest was 2-methyl-2,3-dihydrobenzofuran. A methyldihydrobenzofuran has previously been identified as a marker for *Sphagnum* moss

[46]. The prominence of this compound in the Tomintoul samples provided further evidence that peat from this site contained high levels of *Sphagnum* moss. One sample taken from Hobbister Hill, Htc0, was found to share many chemical characteristics with peat samples from Tomintoul. Of particular note was the unusually high percentage of 2-methyl-2,3-dihydrobenzofuran found in Htc0 suggesting that it may have a high *Sphagnum* moss input. The vegetation at the Hobbister Hill site has previously been described as being mostly *Calluna vulgaris* but in a few wet places *Sphagnum* species dominate [136]. Thus, this sample may represent one of the wetter areas of the site. Indeed, the peat was somewhat shallower at the particular area where transect c was placed.

4.1.3 Peat differentiation due to depth

The sampled peat deposits have been exposed to varying levels of extraction. Whilst at those sites where peat is cut along banks, peat is extracted from all depths simultaneously, at those locations where peat is extruded the extraction depth will obviously change over time. It was important to understand how extraction depth would affect the differentiation of peat due to geographical location. The effect of altering extraction depth was studied at Glenmachrie and Hobbister Hill.

FT-IR analysis showed that samples taken from the upper horizons of depth profiles taken from both Hobbister Hill and Glenmachrie were different from those taken from deeper in the bog. This probably reflects the fact that the samples from the upper horizons contained material that had only relatively recently entered the acrotelm, and as such had not been exposed to the same level of decomposition as the other samples. The underlying samples, on the other hand, are likely to be more similar to each other due to the slowing of decomposition rate as peat passes into the anaerobic catotelm [67]. Peat from the upper regions is not generally used for kilning. Hence the similarity among the remaining samples indicated that depth has relatively little impact on the chemical composition of the peat used industrially.

It must be noted that some of the peat deposits are somewhat deeper than Hobbister Hill and Glenmachrie. Therefore, though analysis of depth profile peat samples in this study showed that peat chemical composition changes little once it has entered the catotelm, it would be of interest to determine whether this finding holds true in much deeper profiles.

The observations made from the pyrolysis work were in agreement with previous studies looking at the peatification process where carbohydrates were found to be preferentially degraded over lignin [45]. Carbohydrate contribution to the Hobbister Hill peat decreased most dramatically in the top section of the depth profile. This change in carbohydrate degradation rate might reflect the transition from aerobic environment, where carbohydrate metabolism is high, to an anaerobic environment where fewer micro-organisms means that carbohydrate degradation would be reduced. A similar pattern of carbohydrate utilisation was seen at Glenmachrie, although the transition from high carbohydrate degradation rate to low degradation rate occurred higher in the depth profile; this may reflect a difference in the size of the aerobic acrotelm at the two sites.

Lignin is known to be recalcitrant [48]. Lignin decomposition takes place primarily in the upper aerobic layer of peat by oxidising fungi. In the anaerobic lower layer lignin undergoes very little decomposition [31]. As such, it was unsurprising to find that the ratio of oxidised lignin to unoxidised lignin, represented by the ratio of acetovanillone to *trans*-isoeugenol, was found to be lower in the surface layer of Hobbister Hill peat, which was relatively more intact than in the underlying peat. In the case of the Glenmachrie depth profile, while the uppermost sample (Lp0) contained slightly lower levels of oxidised lignin than the two samples below (Lp1+2), the oldest sample (Lp3) contained an even lower level of oxidised lignin. It may be that sample Lp3 had undergone less aerobic lignin degradation than Lp1 and Lp2 prior to entering the anaerobic catotelm. Indeed, it was noted that whilst the bottom three Glenmachrie samples all contained less recognisable plant material than sample Lp0, Lp3 appeared to contain some larger more fibrous particles than samples Lp1 and Lp2. Therefore, this variation may be due to differences in the environmental conditions over time which could affect the activity of degrading microorganisms and thus the level of lignin oxidation. The levels of lignin

oxidation were correlated to the levels of nitrogen-containing compounds in samples at both sites. This finding provides further evidence that nitrogen-containing compounds accumulate in lignin degraded peat.

4.1.4 Effect of temperature on peat pyrolysis products

Py-GC-MS analysis was able to show the chemical reasons for the differentiation of peat due to geographical location and sampling depth. In addition to differences in the raw material (peat), another source of variation when using peat for kilning is the kilning process. In this respect, changes in temperature are known to have a major influence on the pyrolysis products of biomass [96]. Using Py-GC-MS, it was possible to determine the effects of varying the temperature on the pyrolysis products of peat.

In the method development section (Chapter 2.4.3) it was observed that pyrolysis temperature has a dramatic effect on the proportions of compounds present in the pyrolysate. Clearly, fluctuations in this parameter could therefore have a major impact on the composition of peat smoke and, consequently, on the composition of peated malt and peated new-make spirit. It is of importance, therefore, to be aware of the need to control peat pyrolysis temperature in order to produce peated malt of the desired composition. Additional work is required in order to fully understand the impact which alterations in peat pyrolysis temperature could have on final spirit quality.

4.2 Peated Malt

Peats from different geographical locations were found to be chemically distinct. The next step, therefore, was to determine if this differentiation was evident in peated malt produced using peat from these locations. Analysis of lab-scale peated malt showed that the source of peat had a direct impact on the malt chemical composition. Additionally, despite the variation in production process, the impact of peat source was also detectable in industrially produced peated malts.

4.2.1 Chemical characterisation of lab-scale peated malts

During the production of peated malt on the industrial scale, variables other than the source of peat may have an impact on the composition of the peated malt. Indeed, from the Py-GC-MS work carried out as part of this study, it was evident that pyrolysis temperature could have a major impact on peated malt composition. For this reason, peated malt was produced on the laboratory scale to eliminate such variables. These lab-scale peated malts were then analysed and their compositions correlated with that of the source peat.

Compounds derived from malt and peat

The initial analysis of all potential peat-derived compounds detected in peated malt showed that several of these compounds were also found in unpeated malt. Some of these malt-derived compounds were present at appreciable levels in the unpeated malt (more than 20% of peated malt levels). These included acetic acid which is known to be produced by the thermal degradation of wood polysaccharides and so may have been formed when malt was heated during kilning [137]. Benzeneacetaldehyde can arise from Strecker degradation of phenylalanine during heating [138]. The origin of a benzyl alcohol was uncertain. Benzoic acid may be an intermediate in the formation of salicylic acid, an immune hormone in plants [139]. Benzeneacetic acid is known to be a natural auxin, or plant growth substance, previously identified in higher plants such as barley [140].

Other peat-derived compounds which were also detected in unpeated malt at lower levels included a group of furans and cyclic ketones probably derived from the thermal degradation of carbohydrates found in malt [141]. Some oxygenated guaiacyl derivatives were also detected at low levels in unpeated malt. The most prominent of these, vanillin, has been previously found free in barley malt [83]. Several nitrogen-containing compounds were detected at very low levels. Compounds belonging to this group have been reported previously in speciality malts and their products at appreciable levels

[142,143]. These speciality malts are, however, kilned at higher temperatures than distillery malt resulting in an increase in the occurrence of Maillard reactions known to produce these compounds [141]. Acetophenone may be derived from leucine during heating [144]. The origin of other peat-derived compounds detected in unpeated malt was unclear.

Marker phenols

Comparing peated malts produced using peat from different sources showed large differences in the levels of these compounds. For example, the level of guaiacol, a compound known to be present at aroma active levels in peated whiskies [14], ranged from 9.6 mg kg^{-1} in Hobbister Hill peated malt to 19.3 mg kg^{-1} in Castlehill peated malt. Therefore, when using the marker phenols to determine the peating level, it can be seen that different peats contribute different ranges of compounds. This fact may be important when selecting peat to be used for peating malt, particularly if peat supplies are limited.

An important compound for differentiating peated malts, in terms of its relative abundance, was *m*-cresol. This compound was relatively abundant in the St Fergus and Tomintoul peated malts. The reasons for this relative abundance are unclear as no data are available concerning the source of this compound in peat. This may be because analysis of this compound in peat is difficult due to its tendency to co-elute from GC columns with *p*-cresol. As it was speculated in Chapter 4.1.1 that the St Fergus and Tomintoul peats were less degraded than the Hobbister Hill and Islay peats, it may be that *m*-cresol is a more prevalent thermal breakdown product from undegraded peat.

There were some differences in the levels of compounds found in different samples made using the same peat. This finding was probably a reflection of the uneven distribution of peat smoke compounds throughout the malt as it was being peated. As a single aliquot of 5 g was taken for analysis from each malt sample (of approximately 400 g), this may not have been a representative sample. Indeed, when three aliquots from each sample were compared this variance was minimised (Table 2.27). This is a phenomenon which has been noted previously in studies of peat smoke applied to malt during kilning [145].

Total chemical profiles

The levels of some compounds (e.g. propylsyringol) showed even more variance between peat sources than was found for the marker phenols. It is unknown if compounds such as this have an impact on spirit flavour, or indeed if they are present in the final spirit. Clearly, if this was found to be the case, then these large variances could be very important for peated whisky production.

When comparing peated malt to peat, while the same classes of compounds could be detected, the exact species of compounds belonging to each class were different. This difference may be due, at least in part, to the different methods used to analyse the two matrices and their different sensitivities. It may also be due to the different thermal breakdown conditions experienced by the peat during anaerobic pyrolysis when performing Py-GC-MS, and during aerobic combustion when malt peating is being carried out. For example, the production of oxidised lignin derivatives is known to be enhanced during aerobic combustion [95]. Also, as in the case of some of the larger lignin-derived compounds identified in peat samples, it may be that some compounds, being relatively non-volatile, do not reach the malt during peating, explaining their absence from peated malt.

Despite the differences in the particular chemical species present, the distribution of most of the chemical classes (syringols, guaiacols, carbohydrate derivatives, nitrogen-containing compounds and, to a large extent, the phenols) could generally be correlated in peat and peated malt. This is because compounds from each of these classes tend to be derived from the same parent structures, e.g. syringols and guaiacols and many phenols are derived from lignin. This correlation was less straightforward for the aromatic compounds. This was due to the different species detected in peat and peated malt and the fact that these compounds may be derived from different parent structures. In the case of polycyclic aromatic hydrocarbons (PAHs) such as the naphthalenes, these compounds were not found to significantly differentiate peats or peated malts. These compounds are usually considered secondary products of pyrolysis reactions and as such

their formation depends not only on the nature of the substrate but also on temperature and residence time in the heated zone [146]. Therefore, these compounds may be of more importance, in terms of differentiating peated malts, when different combustion conditions are encountered.

4.2.2 Comparison of lab-scale and industrial peated malts

The composition of peated malt produced on the laboratory scale was found to be well correlated with the composition of the source peat. On the industrial scale, parameters other than the peat composition may have an impact on peated malt composition. Therefore, it was important to compare lab-scale and industrial peated malts to determine if the differentiation due to peat source could be detected in industrial malts or whether this had been dissipated by the other processing variations.

Marker phenols

The comparison of the composition of laboratory produced peated malt with industrially produced samples showed that, although there were many similarities, the laboratory procedure did not exactly replicate the industrial process. The reason for this may be inferred by observing the relative abundances of the compounds present (Fig. 3.13). In the case of the lab-scale samples, guaiacyl compounds were relatively more abundant whilst in industrial samples the cresols, phenol and 4-ethylphenol were more abundant. This finding could be explained by the peat combustion temperatures. A comparison of the pyrolysis of peat at 610 °C and 770 °C showed that at 610 °C guaiacyl compounds were relatively more abundant while at 770 °C the non-methoxy phenols were more abundant (Fig. 2.13). It may be that the temperatures achieved during the industrial production of peated malts are somewhat higher than those attained in the lab. The maximum temperature of peat combustion in the lab was measured at approximately 600 °C. There are little data available on the temperature of combustion achieved in industrial kilns, but there is some suggestion that temperatures of over 1000 °C are reached. If lab-scale peating could be carried out at different temperatures it may be possible to confirm this speculation.

Total chemical profiles

When the industrial data set was analysed by PCA, the distribution of the industrial samples was not identical to that of the lab-scale samples. This was not surprising, as peating level was found to be the major contributor to industrial peated malt composition and also, as was mentioned in Chapter 1.3.4, there are several possible additional sources of variation in peating methods which could contribute to differences in peat smoke composition. There were however, several similarities between the distributions of the lab-scale and industrial samples. For example, the distinction of samples containing high proportions of oxygenated guaiacyl compounds from those containing high proportions of propenylguaiacols was maintained. This was not expected as it is known that the production of oxygenated lignin derivatives in biomass smoke is influenced by the level of oxygen present and the control of oxygen availability vary markedly from one industrial peating system to the next and are not tightly controlled [95]. That the industrial peated malts showed some similarity to the lab-scale products demonstrated that peat source is an important parameter that can affect peated malt composition irrespective of peating method.

The main difference between lab-scale peated malt and industrial peated malt was that the industrial St Fergus peated malt lacked the elevated levels of lignin-derived phenolics found in the lab-scale samples. The reason for this may relate to the heterogeneous nature of the St Fergus peat. From the distribution of peat samples shown in Fig. 3.6, it was apparent that, though all three St Fergus samples were localised to a distinct cluster, they did not all contain equally high proportions of lignin-derived compounds. It may be, therefore, that the total lignin content of St Fergus peat depends on the area of the deposit from which the peat is extracted. However, the fact that both lab-scale and industrial St Fergus peated malts were similarly differentiated in terms of their marker phenols, both containing relative abundances of methylguaiacol, did suggest a common feature in the lignin composition of the peat used to produce these malts. It was shown by Py-GC-MS in Chapter 3.1.2 that St Fergus peat was relatively abundant in this compound reflecting the common origin of the peat used to produce the industrial and lab-scale peated malts.

4.3 Peated New-Make Spirit

The composition of peated malt was found to be affected by the source of the peat used. Therefore, the next stage in this project was to determine whether this differentiation was maintained in the new-make spirit. Analysing new-make spirits produced using different peats showed that peat source had an impact on the chemical composition of new-make spirit. It was also found that the impact of peat source could be detected in industrially produced new-make spirits. These chemical differences were then correlated with sensory differences showing how peat source affected new-make spirit flavour.

4.3.1 Lab-scale new-make spirit production

The composition of new-make spirit produced on the industrial scale is known to be affected by a number of processing variables [147]. Therefore, as with peated malt, it was important to produce new-make spirit on a laboratory scale in order to control these variables and determine how peat source alone affected new-make spirit composition.

During the development of a lab-scale new-make spirit production method, the effect of changes in some production parameters on the composition of peated new-make spirit was noted. One such parameter found to affect the composition of the spirit, which was described in the method development section, was the efficiency of the mashing pump used. A more efficient pump extracts more sugars into the wort which ultimately results in a higher strength of distillate. The steam volatile phenols (such as the marker phenols) tend to distil at a higher rate towards the end of the spirit cut and into the feints fraction. This is due to the increasing ratio of water to alcohol at this stage of the process which favours the entrainment of the phenols [148]. Given that spirit cuts in the lab-scale spirit production were determined by volume, using a more efficient pump would mean that the spirit would be at a relatively high strength towards the end of the spirit cut and therefore the concentration of phenols in the spirit would be reduced.

It has been found previously that the rate of distillation is an important factor affecting spirit composition [148]. Though not described in this work, it was found during method development that if distillation rate was poorly maintained from distillation to distillation, there was a significant effect on the spirit composition. It is known that the lower the rate of distillation, the higher the strength of distillate obtained. As explained above, this change in the strength was found to affect the concentration of steam volatile phenols in the spirit.

These observations highlighted, with specific reference to peated products, the scope for variations in the new-make spirit production process. Further work is required to determine how the various parameters contributing to the production process can be manipulated in order to optimise the quality of peated spirit produced.

4.3.2 Chemical characterisation of lab-scale peated new-make spirits

A total of 26 phenolic compounds were identified in lab-scale peated new-make spirit using direct injection. This was a similar number to that found in a previous study on industrially produced matured peated whisky [9]. Generally the species of phenols present in the lab-scale new-make spirit and the industrial matured spirit were similar, though in both studies the exact isomers of some compounds were not always known. The same information was not available for the other classes of compounds detected in the lab-scale spirit (carbohydrate derivatives, nitrogen-containing compounds, aromatic compounds). Nevertheless, for all peat-derived compounds, even if the same compounds are present in both new-make and matured spirit, it is unknown if the same levels and proportions of these compounds are maintained throughout the maturation process. This is therefore an area for future studies as maturation could play a role in altering the peat-derived flavour of new-make spirit.

It was observed that the concentrations of marker phenols were much lower in the new-make spirit than in the malt. This is in agreement with previous findings that the majority of phenols are lost to the system during spirit production [9]. This finding may help to

explain the lack of syringyl compounds in new-make spirit, which are present at relatively low levels in the malt. Given their relatively large size and consequent high boiling points, this would make the syringols less likely to distil into the final spirit. Though the syringols themselves were not present in the new-make spirit, there were some compounds present which may be derived from syringyl lignin, and these were the alkyl aryl ethers such as dimethoxytoluene [149].

Analysis of all the peat-derived compounds in lab-scale new-make spirit showed that, despite the decrease in total number of compounds through the production process, the differentiation of samples made using different peats was maintained. Therefore differences in the composition of peat have a direct impact on the chemical composition of peated new-make spirit. It was important to note that new-make spirits could be differentiated not only in terms of the total peat-derived compounds but also in terms of the marker phenols alone. These are compounds known to contribute to spirit flavour and differences in the profile of these compounds are therefore likely to have an impact on flavour. Also, the profile of these marker phenols could potentially be used as an indicator of the broader profile of peat-derived compounds found in spirit.

4.3.3 Comparison of lab-scale and industrially produced peated new-make spirit

As explained previously, there are several processing parameters that can be altered during the production of new-make spirit which can affect the composition of the spirit. Nevertheless, spirit samples produced on an industrial scale could be differentiated according to the source of peat used (Fig. 3.23 and 3.29). As with the lab-scale samples, this differentiation was detected in both the total chemical profiles and the marker phenols profiles. The fact that marker phenols alone could be used to differentiate spirit produced using different peats suggests, as was found in lab-scale samples, that these compounds could be used to distinguish between peat sources.

Analysis of industrially produced peated new-make spirit suggested that peat source has a major influence on spirit composition, given that differences in production methods were

not able to negate its effect. The variance due to production method may be responsible though, for the relatively large differences within spirits produced from the same peat source. Much of this variance will have been due to the amounts of peat used in the production of these spirits but it is likely that variance in other production parameters will have exerted an effect.

4.3.4 Relating sensory and analytical data

An influence of peat source on the chemical composition of peated new-make spirit was established. The final stage in this project showed that these differences in composition have an impact on spirit flavour.

Lab-scale new-make spirits

Lab-scale peated new-make spirits were used to define the links between analytical and sensory data as these the composition of these samples was controlled for all factors other than peat source. Sensory analysis showed that there were differences in the aroma of the spirits made with different peats. PCA of the 46 aroma-active compound peak area data, identified using GC-O/MS, along with the sensory data showed that the phenolic compounds and the alkyl aryl ethers generally co-localised well with the peaty aromas. This is in good agreement with the popular consensus that these compounds are central to smoke flavour [78]. It was also clear that, in addition to the overall levels, the proportions of these compounds had an impact on flavour. This was demonstrated by the unexpectedly low levels of the peaty aromas in the St Fergus spirit, despite a relatively high abundance of peaty aroma compounds. A relative abundance of the guaiacols, particularly those with longer side chains, and the alkyl aryl ethers relative to the phenols in the St Fergus spirit appeared to be responsible. The compounds characterising St Fergus spirit tended to have sweet and spicy aromas, while the phenols had more medicinal aromas. This elevation of sweet and spicy aromas in the St Fergus spirit may therefore have had a negative effect on the impact of the peaty aromas. The presence of guaiacols with longer side chains is thought to indicate relatively undegraded lignin in peat, while non-methoxylated phenols indicate a more degraded peat [45]. The

suggestion is that the St Fergus peat is relatively undegraded and this has resulted in the relatively low levels of peaty aromas in the spirit.

In terms of the balance of peaty aromas, Tomintoul spirit was separated from Hobbister Hill spirit by an elevated level of medicinal aroma in the former. Whilst Tomintoul spirit generally contained more phenols, two phenolic compounds (2-chlorophenol and a hydroxymethylacetophenone) were found to be distinct from the rest and were particularly characteristic of the Tomintoul spirit. In terms of the origin of these compounds, 2-chlorophenol may arise either from anthropogenic sources, herbicides, pesticides or preservatives [150], or they can also be produced by various species of basidomycetous fungi [151]. The origin of the hydroxymethylacetophenone is not clear. It is possible that the increased abundance of this compound is related to the high level of *Sphagnum* moss in Tomintoul peat. Certainly though, the presence of a relatively high level of *Sphagnum* does seem to confer an elevated level of medicinal aroma.

Many of the identified aroma compounds, outwith the phenolic compounds, detected by GC-O/MS did not appear to contribute directly to the peaty aromas analysed in this study. That is not to say that they do not contribute to the overall aroma of the spirit. For instance, the higher level of grassy aroma in Hobbister Hill spirit may be due to one or more of these compounds though the identity of the compound or compounds responsible would require further investigation.

It must also be noted that there were 33 peat-derived aromas identified by GC-O/MS which had no chromatographic peak associated with them and eight of these had dilution values of 10 or more. It would therefore be of interest to identify the compounds responsible and correlate their abundance with sensory data to indicate what extent they contribute to peaty aromas.

Industrial samples

It was important to compare the lab-scale peated new-make spirit with industrial equivalents in order to ascertain how relevant the findings made from the lab-scale samples would be to the industrial product.

The comparison of lab-scale and industrial samples by GC-O/MS showed that there were a number of additional aromas in the lab-scale samples. The aromas of these compounds were generally not described as typical peaty aromas. As such, these compounds were not of central interest to this study. Those compounds that did have peaty aroma descriptors were predominantly the guaiacols, phenols and the alkyl aryl ethers. In terms of these compounds, the lab-scale and industrial samples were qualitatively closely matched. Therefore, the compounds being analysed in lab-scale samples were relevant to the industrial product.

It has been mentioned previously, though, that the proportions of individual compounds in lab-scale and industrial samples are different. Indeed, using Quantitative Descriptive Analysis, it was possible to relate the different proportions of peat-derived compounds in industrial and lab-scale spirits to differences in the peat-derived aroma (Fig. 3.32). Industrial peated new-make spirit tended to have a relatively medicinal aroma whilst the lab-scale new-make spirit had more of a burnt and smoky aroma. GC-MS analysis showed that phenols were relatively more abundant in the industrial samples whilst guaiacols were more abundant in the lab-scale samples. GC-O/MS analysis of these compounds showed that phenols tended to have more medicinal type aromas whilst the guaiacols tended to have more burnt and smoky type aromas. The disparity in the chemical composition of lab-scale and industrial spirit is no doubt due to differences in the production process carried out in the lab compared with the industry. This suggests that it may be possible to manipulate the production process in order to influence the composition, and ultimately, the flavour of peated new-make spirit.

4.4 Conclusions and Future Work

Through the application of FT-IR spectroscopy and Py-GC-MS, this study has demonstrated compositional differences in the peat used by the Scotch whisky industry depending on its geographical source. By producing peated malt and new-make spirit on a laboratory scale, it was possible to show that the chemical differences between peat from different sources were maintained through to the final product. Furthermore, several of the chemical differences detected in laboratory produced malt and spirit could also be detected in the industrially produced equivalents indicating the high level of influence that peat source has on chemical composition. These differences were transferred to new-make spirit where peat source was found to have a significant influence on flavour. The compounds responsible for the peaty flavour of new-make spirit were found to predominantly be a spectrum of phenols and guaiacols.

Data obtained over the course of this work indicated how variance in some production parameters, such as peat pyrolysis temperature during kilning, could affect the chemical composition of peated new-make spirit. Therefore, future work should be carried out to discern what the effects of alterations in parameters such as peat pyrolysis temperature have on the flavour of new-make spirit. It may be possible to manipulate production parameters to alter the flavour of the spirit. Also, the impact of maturation on the composition of peated new-make spirit should be investigated in order to ascertain how this process, known to have a large impact on whisky flavour [152], affects the flavour differences detected in peated new-make spirits produced using different peat.

Appendix A

Table 2.6. Compounds detected by Py- GC-MS of peat samples.

Peak no. [a]	Compound name	RT [b]	Estimated RI [c]	Literature RI [d]	QI [e]	MW [f]	Source [g]
1	2-Methylfuran	2.13	656	608	82	82	Ps
2	Acetic acid	2.25	660	**618**	60	60	Xyl
3	*trans*- 2-Butenal	2.67	677	626	70	70	Ps
4	3-Methylbutanal	2.76	680	657	86	86	
5	Benzene	2.81	682	**656**	78	78	
6	2,3-Pentanedione	3.31	701		100	100	Ps
7	2-Ethylfuran	3.36	703	707	81	96	
8	2,5-Dimethylfuran	3.46	707	713	96	96	Ps
9	2,3-Dimethylfuran	3.62	713	725	96	96	Ps
10	Vinylfuran	3.78	719	718*	94	94	Ps
11	1-Methyl-1H-pyrrole	4.07	731	751	81	81	Ext
12	Pyridine	4.25	737	**746**	79	79	
13	Pyrrole	4.32	740	**757**	67	67	Ext
14	Toluene	4.71	755	**767**	91	92	
15	(2H)-Furan-3-one*	5.42	783	763	84	84	Hex
16	3-Furaldehyde	5.81	798	817	95	96	Ps
17	2-Methylpyridine	5.92	802	823	66	93	
18	Furfural	6.28	816	**829**	96	96	Ps
19	1-Cyclopentene-3,4-dione	6.42	821	849	96	96	Hex
20	Ethylbenzene	7.07	846	**868**	91	106	Lg, biod
21	Methylpyridine 2	7.11	848		66	93	
22	Acetol acetate	7.15	849	867	116	116	Xyl
23	5-Methyl-2(3H)-furanone	7.19	851	903	98	98	Hex
24	Xylene 1	7.31	855		91	106	
25	Cyclopentenedione 2	7.58	866		96	96	Hex
26	Cyclopentenedione 3	7.68	870		96	96	Hex
27	Styrene	7.92	879	**895**	104	104	
28	Xylene 2	7.95	880		91	106	
29	2-Methyl-2-cyclopentene-1-one	8.25	892	910	67	96	Ps
30	(5H)-Furan-2-one	8.33	895	870	55	84	Hex
31	2-Acetylfuran	8.50	901	907	110	110	Ps
32	Methoxybenzene	8.61	905	919	108	108	
33	2-Hydroxy-2-cyclopenten-1-one	8.82	914		98	98	

Appendix A

Peak no. [a]	Compound name	RT [b]	Estimated RI [c]	Literature RI [d]	QI [e]	MW [f]	Source [g]
34	5-Methyl-2(5H)-furanone	9.10	924		55	98	Hex
35	3-Methyl-2,5-furandione	9.26	930		68	112	
36	5-Methylfurfural	9.86	954	**968**	110	110	Ps
37	2H-Pyran-2-one	10.07	962	945	68	96	
38	3-Methyl-2(5H)-furanone	10.19	966		41	98	Hex
39	Phenol	10.34	972	**984**	94	94	
40	Benzofuran	10.95	996	1001	118	118	
41	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	11.05	999	967*	114	114	Pent
42	3-Hydroxy-2-methyl-2-cyclopentene-1-one	11.41	1013	1002	112	112	Hex
43	Cyclotene	11.71	1025	1023	112	112	Hex
44	2-Acetyl-5-methylfuran	11.93	1033	1014	109	124	Ps
45	2,3-Dimethyl-2-cyclopenten-1-one	12.01	1036	1037	67	110	
46	4-Methyl-5H-furan-2-one	12.14	1041		69	98	Hex
47	Indene	12.35	1050	1053	115	116	
48	*o*-Cresol	12.44	1053	1055	108	108	Pp
49	2-Pyridinecarbonitrile	12.74	1065	1079	104	104	
50	Acetophenone	12.93	1072	**1063**	105	120	Lg, biod
51	*m-/p*-Cresol	13.05	1076	1079 (*m*-), 1078 (*p*-)	107	108	Pp
52	Methylfuroate	13.32	1087		95	126	Ps
53	Guaiacol	13.46	1092	**1085**	109	124	Lg
54	Maltol	14.14	1118	1098	126	126	Hex
55	2-methyl-5-hydroxy-(4H)-pyran-4-one	14.70	1140		126	126	Ps
56	2-Ethylphenol	14.74	1142	1148	107	122	Pp
57	Benzyl nitrile	14.92	1149	1141	117	117	Pr
58	Dimethylphenol 1	15.08	1155		107	122	Lg, biod
59	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	15.09	1155	1134	144	144	
60	Dimethylphenol 2	15.14	1157		107	122	Lg, biod
61	3-Methyl-1H-indene	15.27	1162		115	130	
62	4-Ethyl-1,2-dimethylbenzene	15.31	1164		119	134	
63	4-Ethylphenol	15.54	1172	1167	107	122	Pp
64	Benzoic acid	15.66	1177	1214	105	122	
65	4-Methoxy-3-methylphenol	15.92	1187		123	138	
66	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	16.22	1199	1162	142	142	

Appendix A

Peak no. [a]	Compound name	RT [b]	Estimated RI [c]	Literature RI [d]	QI [e]	MW [f]	Source [g]
67	Methylguaiacol	16.27	1201	**1188**	123	138	Lg
68	Pyrocatechol	16.34	1203	1182	110	110	Pp
69	2,3-Dihydrobenzofuran	16.70	1217	1224	120	120	
70	Dihydrobenzofuran 2	16.96	1227		120	120	
71	Unknown phenolic 1	17.22	1237		110		
72	5-Hydroxymethylfurfural	17.25	1238	1240	97	126	Ps
73	Ethylmethylphenol	17.44	1246		121	136	Pp
74	2-Coumaranone	17.57	1251		134	134	
75	Propenylphenol 1	17.84	1261		134	134	Lg
76	4-Propylphenol	17.96	1266		136	136	Pp
77	Methylbenzenediol	17.96	1266		124	124	Pp
78	3-Methoxy-1,2-benzenediol	18.05	1269		140	140	Lg
79	Ethylguaiacol	18.46	1285	**1281**	137	152	Lg
80	Indole	19.00	1306	**1301**	117	117	
81	2-Methyl-2,3-dihydrobenzofuran	19.08	1309		134	134	Sphag
82	2-Methylnaphthalene	19.21	1314	1293	142	142	
83	Vinylguaiacol	19.43	1322	**1315**	150	150	Lg
84	1,3-Dimethoxybenzene	19.53	1326	1284	138	138	
85	1-Methylnaphthalene	19.61	1329	1309	142	142	
86	Propenylphenol 2	20.06	1347		134	134	Lg
87	Syringol	20.25	1354	**1345**	154	154	Lg
88	Eugenol	20.41	1360	**1357**	164	164	Lg
89	4-Butylphenol	20.50	1363		107	150	
90	Propylguaiacol	20.65	1369	**1356**	137	166	Lg
91	4-Ethylcatechol	21.00	1383		123	138	
92	3-Methyl-1H-indole	21.30	1394	1403	130	131	Pr
93	Vanillin	21.51	1402	**1406**	151	152	Lg
94	*cis*-Isoeugenol	21.64	1407	1395	164	164	Lg
95	4-Acetylphenol	22.43	1438	1442	121	136	
96	Methylsyringol	22.46	1439	**1413**	168	168	Lg
97	*trans*-Isoeugenol	22.66	1447	1465	164	164	Lg
98	Unknown guaiacyl isomer 1	23.39	1475		147		Lg
99	Acetovanillone	23.49	1479	**1481**	151	166	Lg
100	Unknown guaiacyl isomer 2	23.51	1479		147		Lg
101	Unknown phenolic 2	23.61	1483		166		Lg
102	Methylvanillate	24.13	1503	1511	151	182	

Appendix A

Peak no. [a]	Compound name	RT [b]	Estimated RI [c]	Literature RI [d]	QI [e]	MW [f]	Source [g]
103	Ethylsyringol	24.18	1505	**1496**	167	182	Lg
104	Guaiacylacetone	24.38	1513	1513	137	180	Lg
105	Dibenzofuran	24.46	1516	1511	168	168	
106	Vinylsyringol	25.10	1541	**1552**	180	180	Lg
107	Vanillic acid	25.21	1545	**1546**	168	168	
108	Guaiacyl propanaldehyde	25.58	1559	1581*	180	180	Lg
109	Propylsyringol	25.98	1575	**1583**	167	196	Lg
110	Methoxyeugenol	26.86	1608	1601	194	194	Lg
111	Homovanillic acid	26.92	1611	1633	137	182	Lg
112	Syringaldehyde	27.11	1618	**1645**	182	182	Lg
113	Unknown syringyl monomer 1	27.60	1637		192		Lg
114	Unknown syringyl monomer 2	27.73	1642		192		Lg
115	Propenylsyringol	27.94	1650		194	194	Lg
116	Acetosyringone	28.54	1673	1720	181	196	Lg
117	Coniferaldehyde	28.74	1681	1726*	178	178	
118	Syringylpropanone	29.17	1697	1755*	167	210	Lg
119	Syringic acid	30.13	1734	1793*	198	198	
120	Syringyl propanaldehyde	30.28	1740	1852	210	210	Lg
121	Ferulic acid	31.25	1778		194	194	
122	Dimethoxycinnamic acid	32.44	1823		208	208	

[a] Colour coding indicates class of compound: carbohydrate-derived (identified using data from van Smeerdijk et al.), guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] RT = Retention Time (minutes).

[c] RI = Retention Index.

[d] Literature RI data taken from [149,153-157]. Estimated RI were calculated as described in Chapter 2.4.2 using literature RI data for selected compounds (identified in bold). Compounds marked with a * were identified by RI alone.

[e] QI = Quantitation Ion.

[f] MW = Molecular Weight.

[g] Source data taken from [46]. Abbreviations: Ps = polysaccharides; Hex = hexosan; Pent = pentosan; Rham = rhamnose; Xyl = xylan; Pr = protein; Ext = extension; Pp = polyphenols; Lg = lignin; Lg, BIOD = degradation product of lignin; Sphag = characteristic compound for *Sphagnum*.

Appendix B

Table 2.8. Py-GC-MS normalised peak area data for compounds significantly differentiating peat pyrolysed at different temperatures.

Peak no.[a]	Compound name	Pyrolysis temperature (°C)			
		358	510	610	770
1	2-Methylfuran	0.48	1.04	0.84	0.82
2	Acetic acid	4.56	5.71	4.68	3.51
3	*Trans*-2-Butenal	0.11	0.12	0.22	0.30
4	3-Methylbutanal	0.01	0.03	0.03	0.03
5	Benzene	0.39	0.65	1.36	3.45
7	2-Ethylfuran	0.02	0.08	0.10	0.16
8	2,5-Dimethylfuran	0.04	0.27	0.27	0.27
9	2,3-Dimethylfuran	0.06	0.11	0.12	0.12
10	Vinylfuran	0.10	0.20	0.17	0.20
11	1-Methyl-1H-pyrrole	0.08	0.22	0.27	0.24
12	Pyridine	0.42	0.88	0.85	0.91
13	Pyrrole	0.32	0.79	0.87	1.09
14	Toluene	0.13	1.18	3.37	5.79
15	(2H)-Furan-3-one	5.93	5.52	3.22	2.64
17	2-Methylpyridine	0.01	0.06	0.10	0.13
18	Furfural	11.30	6.60	4.11	3.95
19	1-Cyclopentene-3,4-dione	2.26	1.02	0.46	0.34
20	Ethylbenzene	0.02	0.11	0.47	0.84
21	Methylpyridine 2	0.04	0.10	0.10	0.11
24	Xylene 1	0.10	0.24	0.64	1.23
27	Styrene	0.03	0.28	0.70	1.21
28	Xylene 2	0.00	0.05	0.37	0.50
29	2-Methyl-2-cyclopentene-1-one	0.03	0.12	0.27	0.37
30	(5H)-Furan-2-one	0.48	0.83	0.76	0.79
31	2-Acetylfuran	0.33	0.14	0.13	0.14
32	Methoxybenzene	0.01	0.02	0.08	0.10
34	5-Methyl-2(5H)-furanone	0.17	0.32	0.18	0.17
35	3-Methyl-2,5-furandione	0.33	0.55	0.40	0.25
36	5-Methylfurfural	2.72	1.95	1.40	1.64
37	2H-Pyran-2-one	0.05	0.11	0.15	0.21
39	Phenol	0.56	2.85	7.76	12.69
40	Benzofuran	0.01	0.04	0.15	0.33
41	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	17.45	7.64	4.26	2.48
43	Cyclotene	0.19	0.55	0.60	0.61

Appendix B

Peak no. [a]	Compound name	Pyrolysis temperature (°C)			
		358	510	610	770
44	2-Acetyl-5-methylfuran	0.04	0.08	0.10	0.11
45	2,3-Dimethyl-2-cyclopenten-1-one	0.01	0.08	0.17	0.18
46	4-Methyl-5H-furan-2-one	0.03	0.25	0.24	0.19
47	Indene	0.01	0.00	0.28	0.56
48	*o*-Cresol	0.01	0.08	0.62	1.27
49	2-Pyridinecarbonitrile	0.00	0.01	0.04	0.10
50	Acetophenone	0.24	0.34	0.34	0.38
51	*m-/p*-Cresol	0.09	1.41	4.46	6.36
52	Methylfuroate	1.00	0.89	0.45	0.29
53	Guaiacol	0.42	2.43	3.30	2.18
54	Maltol	2.49	1.33	1.11	0.82
55	2-methyl-5-hydroxy-(4H)-pyran-4-one	0.67	0.50	0.31	0.20
56	2-Ethylphenol	0.01	0.01	0.10	0.17
57	Benzyl nitrile	0.00	0.12	0.23	0.35
58	Dimethylphenol 1	0.01	0.04	0.31	0.53
59	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	0.66	0.52	0.24	0.10
60	Dimethylphenol 2	0.01	0.03	0.13	0.24
61	3-Methyl-1H-indene	0.01	0.01	0.10	0.11
62	4-Ethyl-1,2-dimethylbenzene	0.01	0.05	0.13	0.15
63	4-Ethylphenol	0.11	0.58	1.51	2.34
64	Benzoic acid	0.02	0.10	0.08	0.05
66	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	1.62	2.85	1.26	0.61
67	Methylguaiacol	0.11	1.65	2.40	1.41
68	Pyrocatechol	0.04	0.93	4.72	3.65
69	2,3-Dihydrobenzofuran	0.00	0.01	0.12	0.39
70	Dihydrobenzofuran 2	10.28	9.89	7.44	6.82
71	Unknown phenolic 1	0.01	0.04	0.28	0.66
72	5-Hydroxymethylfurfural	0.17	1.15	0.73	0.58
73	Ethylmethylphenol	0.01	0.03	0.15	0.23
74	2-Coumaranone	0.02	0.08	0.10	0.12
75	Propenylphenol 1	0.04	0.07	0.09	0.12
77	Methylbenzenediol	0.01	0.01	0.64	0.55
78	3-Methoxy-1,2-benzenediol	0.06	0.66	1.02	0.50
79	Ethylguaiacol	0.14	0.80	1.27	0.78
80	Indole	0.29	0.45	0.33	0.34
81	2-Methyl-2,3-dihydrobenzofuran	0.12	0.14	0.16	0.18
82	2-Methylnaphthalene	0.01	0.01	0.11	0.27
83	Vinylguaiacol	7.70	9.16	6.75	4.56

Peak no.[a]	Compound name	Pyrolysis temperature (°C)			
		358	510	610	770
84	1,3-Dimethoxybenzene	0.00	0.06	0.22	0.20
85	1-Methylnaphthalene	0.01	0.01	0.08	0.17
86	Propenylphenol 2	0.18	0.26	0.29	0.29
87	Syringol	0.30	0.90	0.92	0.58
88	Eugenol	0.22	0.32	0.29	0.22
89	4-Butylphenol	0.00	0.02	0.26	0.23
90	Propylguaiacol	0.00	0.09	0.19	0.13
91	4-Ethylcatechol	0.01	0.14	0.77	0.61
92	3-Methyl-1H-indole	0.04	0.20	0.27	0.27
93	Vanillin	3.87	2.29	1.70	1.35
94	*cis*-Isoeugenol	0.13	0.23	0.23	0.18
96	Methylsyringol	0.13	0.82	0.83	0.50
97	*trans*-Isoeugenol	1.67	1.74	1.25	0.91
98	Unknown guaiacyl isomer 1	0.93	0.34	0.32	0.25
99	Acetovanillone	3.46	4.25	3.26	2.55
101	Unknown phenolic 2	0.03	0.17	0.16	0.10
102	Methylvanillate	0.18	0.29	0.25	0.19
103	Ethylsyringol	0.04	0.22	0.22	0.13
104	Guaiacylacetone	1.18	1.22	0.80	0.50
105	Dibenzofuran	0.03	0.01	0.07	0.38
106	Vinylsyringol	1.61	2.08	1.33	0.93
107	Vanillic acid	0.64	0.77	0.48	0.20
108	Guaiacyl propanaldehyde	0.07	0.16	0.16	0.12
109	Propylsyringol	0.02	0.05	0.06	0.04
110	Methoxyeugenol	0.21	0.25	0.20	0.14
111	Homovanillic acid	0.12	0.12	0.13	0.04
112	Syringaldehyde	1.15	0.41	0.35	0.31
113	Unknown syringyl monomer 1	1.06	0.43	0.34	0.26
114	Unknown syringyl monomer 2	0.29	0.21	0.14	0.10
115	Propenylsyringol	2.40	1.83	1.11	0.73
116	Acetosyringone	1.49	1.64	1.21	0.81
117	Coniferaldehyde	1.53	0.07	0.06	0.04
118	Syringylpropanone	1.74	1.16	0.67	0.40
120	Syringyl propanaldehyde	0.07	0.13	0.09	0.06

[a] Colour coding indicates class of compound: carbohydrate-derived (identified using data from van Smeerdijk et al.), guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

Appendix C

Table 3.2. Py-GC-MS peak area data for compounds significantly differentiating peat from different geographical locations (Islay, Orkney, St Fergus and Tomointoul).

Peak no. [a]	Compound	Islay [b]	Orkney [b]	St Fergus [b]	Tomintoul [b]
1	2-Methylfuran	0.77 (12.64)	1.19 (5.16)	0.74 (26.87)	1.35 (8.47)
2	Acetic acid	4.06 (5.65)	5.13 (5.39)	3.78 (8.35)	4.30 (4.81)
3	*trans*-2-Butenal	0.19 (11.47)	0.32 (6.77)	0.21 (25.16)	0.33 (12.05)
4	3-Methylbutanal	0.02 (12.25)	0.03 (17.50)	0.02 (6.84)	0.02 (7.14)
5	Benzene	1.13 (21.15)	1.52 (43.66)	0.90 (25.36)	1.06 (16.94)
6	2,3-Pentanedione	0.02 (13.68)	0.03 (19.42)	0.02 (29.85)	0.03 (12.26)
7	2-Ethylfuran	0.08 (10.12)	0.13 (6.98)	0.09 (13.01)	0.14 (10.54)
8	2,5-Dimethylfuran	0.26 (18.42)	0.36 (9.55)	0.21 (26.35)	0.39 (14.58)
9	2,3-Dimethylfuran	0.10 (8.78)	0.16 (9.01)	0.15 (31.62)	0.17 (5.94)
10	Vinylfuran	0.14 (8.33)	0.25 (7.53)	0.13 (16.08)	0.33 (12.58)
11	1-Methyl-1H-pyrrole	0.21 (9.97)	0.20 (12.39)	0.27 (26.66)	0.13 (7.90)
12	Pyridine	0.86 (13.96)	0.76 (41.24)	0.69 (20.03)	0.22 (53.15)
13	Pyrrole	0.98 (16.32)	0.92 (23.89)	0.93 (20.23)	0.47 (24.63)
14	Toluene	2.98 (6.85)	3.24 (22.14)	2.23 (8.12)	1.96 (15.04)
15	(2H)-Furan-3-one	2.93 (9.68)	5.19 (7.71)	2.26 (29.99)	6.74 (8.40)
16	3-Furaldehyde	0.48 (13.26)	0.91 (18.61)	0.49 (13.57)	0.94 (28.19)
17	2-Methylpyridine	0.10 (15.75)	0.08 (40.38)	0.09 (43.22)	0.03 (94.86)
18	Furfural	3.60 (4.70)	6.06 (8.69)	3.55 (24.86)	7.95 (6.52)
19	1-Cyclopentene-3,4-dione	0.39 (11.88)	0.54 (14.52)	0.28 (23.04)	0.62 (10.63)
20	Ethylbenzene	0.40 (8.25)	0.45 (29.54)	0.32 (8.43)	0.27 (13.54)
21	Methylpyridine 2	0.14 (14.88)	0.08 (52.78)	0.09 (56.34)	0.01 (70.21)

Peak no. [a]	Compound	Islay [b]	Orkney [b]	St Fergus [b]	Tomintoul [b]
22	Acetol acetate	0.03 (12.94)	0.05 (24.78)	0.03 (35.43)	0.04 (16.33)
23	5-Methyl-2(3H)-furanone	0.24 (8.46)	0.43 (5.07)	0.26 (19.34)	0.45 (10.35)
25	Cyclopentenedione 2	0.28 (5.43)	0.49 (10.51)	0.30 (11.66)	0.49 (8.42)
26	Cyclopentenedione 3	0.23 (14.57)	0.43 (13.59)	0.25 (29.07)	0.42 (11.38)
27	Styrene	0.71 (16.08)	0.89 (34.62)	0.53 (20.30)	0.51 (20.57)
28	Xylene 2	0.31 (17.05)	0.41 (42.11)	0.25 (18.10)	0.26 (14.87)
29	2-Methyl-2-cyclopentene-1-one	0.24 (5.90)	0.35 (10.39)	0.28 (4.35)	0.30 (6.04)
30	(5H)-Furan-2-one	0.91 (6.36)	1.38 (12.82)	0.95 (24.53)	1.52 (8.08)
31	2-Acetylfuran	0.11 (11.71)	0.27 (33.58)	0.11 (15.22)	0.33 (17.65)
32	Methoxybenzene	0.07 (22.02)	0.04 (42.70)	0.04 (23.93)	0.03 (36.36)
33	2-Hydroxy-2-cyclopenten-1-one	1.53 (5.06)	2.35 (7.42)	1.45 (39.06)	2.44 (17.28)
34	5-Methyl-2(5H)-furanone	0.24 (28.60)	0.40 (19.13)	0.23 (20.13)	0.41 (27.63)
35	3-Methyl-2,5-furandione	0.38 (4.87)	0.67 (8.00)	0.39 (15.39)	0.55 (6.93)
36	5-Methylfurfural	1.28 (15.92)	2.65 (20.79)	1.29 (26.55)	3.84 (12.08)
37	2H-Pyran-2-one	0.17 (23.10)	0.29 (32.82)	0.16 (19.22)	0.31 (13.44)
38	3-Methyl-2(5H)-furanone	0.22 (18.71)	0.30 (27.88)	0.19 (28.55)	0.26 (15.47)
39	Phenol	8.40 (16.60)	8.11 (10.96)	5.96 (12.22)	7.79 (8.68)
41	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	3.50 (29.20)	3.76 (34.26)	2.67 (46.90)	4.73 (4.05)
42	3-Hydroxy-2-methyl-2-cyclopentene-1-one	1.46 (12.17)	2.74 (27.86)	1.65 (28.97)	4.07 (14.16)
43	Cyclotene	0.55 (11.66)	0.99 (16.51)	0.55 (25.78)	1.03 (17.08)
44	2-Acetyl-5-methylfuran	0.10 (8.35)	0.15 (11.26)	0.09 (16.07)	0.15 (5.94)
45	2,3-Dimethyl-2-cyclopenten-1-one	0.14 (5.17)	0.20 (7.20)	0.16 (3.20)	0.16 (6.20)
46	4-Methyl-5H-furan-2-one	0.28 (13.34)	0.47 (7.75)	0.27 (25.50)	0.45 (13.16)
47	Indene	0.23 (16.94)	0.34 (41.07)	0.20 (15.32)	0.21 (15.54)
49	2-Pyridinecarbonitrile	0.05 (28.29)	0.06 (28.58)	0.07 (27.34)	0.04 (37.85)
50	Acetophenone	0.28 (19.68)	0.35 (42.31)	0.20 (10.22)	0.27 (16.18)

Peak no. [a]	Compound	Islay [b]	Orkney [b]	St Fergus [b]	Tomintoul [b]
51	*m-/p*-Cresol	4.15 (4.70)	3.43 (7.42)	3.90 (15.36)	3.49 (11.25)
52	Methylfuroate	0.63 (22.87)	1.07 (6.48)	0.47 (25.56)	1.27 (16.34)
53	Guaiacol	3.26 (3.82)	2.47 (21.43)	3.57 (11.45)	1.75 (18.40)
54	Maltol	1.11 (24.66)	2.25 (6.98)	1.11 (29.69)	2.10 (12.04)
55	2-methyl-5-hydroxy-(4H)-pyran-4-one	0.37 (55.33)	0.83 (27.21)	0.22 (45.83)	0.48 (17.67)
56	2-Ethylphenol	0.09 (6.98)	0.10 (19.99)	0.07 (9.52)	0.07 (5.70)
57	Benzyl nitrile	0.24 (8.82)	0.22 (19.38)	0.17 (14.94)	0.12 (24.78)
58	Dimethylphenol 1	0.31 (6.56)	0.26 (9.08)	0.31 (15.03)	0.22 (11.85)
59	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	0.22 (21.79)	0.36 (15.51)	0.12 (52.01)	0.41 (20.43)
60	Dimethylphenol 2	0.09 (21.66)	0.11 (31.49)	0.14 (11.07)	0.09 (11.57)
61	3-Methyl-1H-indene	0.06 (16.67)	0.08 (36.36)	0.05 (16.90)	0.05 (16.12)
62	4-Ethyl-1,2-dimethylbenzene	0.05 (21.84)	0.08 (28.49)	0.08 (29.23)	0.07 (17.45)
63	4-Ethylphenol	1.26 (6.88)	1.00 (27.70)	1.48 (23.18)	1.04 (15.55)
65	4-Methoxy-3-methylphenol	0.14 (11.12)	0.10 (25.05)	0.17 (20.13)	0.07 (14.43)
66	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	1.28 (25.04)	2.02 (10.65)	0.86 (70.23)	2.84 (25.26)
67	Methylguaiacol	2.27 (9.08)	1.47 (7.22)	2.94 (11.95)	1.49 (22.27)
70	Dihydrobenzofuran 2	8.15 (14.20)	4.93 (19.59)	3.54 (35.85)	4.16 (6.87)
71	Unknown phenolic 1	0.39 (31.12)	0.21 (17.33)	0.56 (7.05)	0.26 (24.71)
72	5-Hydroxymethylfurfural	1.22 (33.28)	2.47 (47.54)	1.23 (61.42)	4.49 (8.60)
73	Ethylmethylphenol	0.14 (3.00)	0.11 (9.83)	0.14 (17.22)	0.09 (9.90)
75	Propenylphenol 1	0.07 (15.11)	0.05 (12.17)	0.08 (11.34)	0.05 (10.15)
76	4-Propylphenol	0.03 (22.34)	0.02 (62.11)	0.05 (44.74)	0.01 (93.06)
77	Methylbenzenediol	0.94 (18.65)	0.68 (30.53)	1.22 (7.59)	0.66 (18.00)
78	3-Methoxy-1,2-benzenediol	1.11 (6.77)	0.64 (11.67)	1.36 (29.69)	0.45 (19.50)
79	Ethylguaiacol	1.23 (11.00)	0.79 (9.25)	1.41 (6.01)	0.76 (17.01)

Peak no. [a]	Compound	Islay [b]	Orkney [b]	St Fergus [b]	Tomintoul [b]
80	Indole	0.44 (26.16)	0.31 (49.79)	0.18 (43.36)	0.13 (86.92)
81	2-Methyl-2,3-dihydrobenzofuran	0.17 (18.94)	0.34 (132.06)	0.19 (59.39)	0.61 (40.46)
83	Vinylguaiacol	6.98 (12.66)	4.39 (16.46)	5.91 (11.78)	3.62 (11.22)
84	1,3-Dimethoxybenzene	0.20 (9.88)	0.14 (15.66)	0.27 (27.43)	0.10 (22.81)
86	Propenylphenol 2	0.26 (22.98)	0.17 (23.00)	0.24 (6.39)	0.17 (11.73)
87	Syringol	1.01 (9.98)	0.59 (9.74)	1.67 (33.24)	0.44 (10.80)
88	Eugenol	0.26 (5.99)	0.18 (22.09)	0.59 (22.60)	0.16 (22.69)
89	4-Butylphenol	0.01 (159.58)	0.01 (120.67)	0.38 (81.07)	0.01 (142.09)
90	Propylguaiacol	0.18 (5.15)	0.12 (11.01)	0.29 (15.22)	0.11 (22.15)
91	4-Ethylcatechol	1.31 (22.71)	0.65 (36.15)	1.51 (6.14)	0.71 (12.68)
92	3-Methyl-1H-indole	0.39 (14.23)	0.29 (29.43)	0.20 (22.58)	0.14 (33.36)
93	Vanillin	1.58 (10.41)	1.15 (19.00)	1.96 (30.49)	0.94 (28.21)
94	*cis*-Isoeugenol	0.21 (6.27)	0.14 (18.38)	0.45 (20.68)	0.12 (23.54)
95	4-Acetylphenol	0.97 (6.46)	0.72 (41.21)	0.66 (12.70)	0.81 (11.70)
96	Methylsyringol	0.93 (15.04)	0.48 (22.24)	1.98 (41.94)	0.49 (13.62)
97	*trans*-Isoeugenol	1.16 (5.75)	0.79 (21.54)	2.45 (18.54)	0.69 (21.06)
98	Unknown guaiacyl isomer 1	0.26 (27.34)	0.17 (13.07)	0.56 (46.10)	0.15 (40.71)
99	Acetovanillone	2.88 (12.25)	2.23 (25.57)	3.01 (24.83)	1.37 (23.83)
100	Unknown guaiacyl isomer 2	0.16 (22.35)	0.11 (15.07)	0.39 (43.16)	0.09 (32.63)
101	Unknown phenolic 2	0.22 (23.52)	0.08 (18.89)	0.32 (32.84)	0.07 (43.56)
102	Methylvanillate	0.20 (13.15)	0.16 (35.99)	0.30 (53.86)	0.06 (32.46)
103	Ethylsyringol	0.24 (9.15)	0.13 (21.83)	0.52 (40.18)	0.12 (16.28)
104	Guaiacylacetone	0.78 (7.65)	0.56 (5.94)	1.08 (19.98)	0.45 (22.47)
106	Vinylsyringol	1.63 (9.80)	0.78 (29.17)	3.04 (31.19)	0.70 (21.58)
107	Vanillic acid	1.39 (10.05)	0.53 (73.03)	0.92 (47.05)	0.43 (39.09)
108	Guaiacyl propanaldehyde	0.15 (13.14)	0.10 (22.01)	0.18 (24.13)	0.07 (21.21)

Peak no. [a]	Compound	Islay [b]	Orkney [b]	St Fergus [b]	Tomintoul [b]
109	Propylsyringol	0.08 (15.69)	0.03 (33.36)	0.16 (37.35)	0.03 (24.90)
110	Methoxyeugenol	0.23 (14.58)	0.11 (30.16)	0.46 (32.14)	0.08 (13.58)
111	Homovanillic acid	0.15 (14.02)	0.14 (18.54)	0.48 (29.25)	0.15 (37.87)
112	Syringaldehyde	0.33 (24.43)	0.23 (14.28)	1.27 (48.45)	0.20 (24.65)
113	Unknown syringyl monomer 1	0.34 (28.09)	0.16 (25.73)	0.75 (38.42)	0.13 (44.28)
114	Unknown syringyl monomer 2	0.15 (27.50)	0.06 (44.62)	0.35 (45.04)	0.05 (55.89)
115	Propenylsyringol	1.38 (21.74)	0.61 (36.39)	2.42 (29.39)	0.47 (13.48)
116	Acetosyringone	1.11 (8.79)	0.72 (13.09)	1.97 (36.20)	0.48 (19.66)
118	Syringylpropanone	0.71 (12.13)	0.42 (18.08)	0.89 (27.07)	0.31 (9.05)
119	Syringic acid	0.39 (5.29)	0.11 (75.74)	0.38 (65.51)	0.08 (71.14)
120	Syringyl propanaldehyde	0.10 (24.51)	0.05 (27.98)	0.13 (35.13)	0.03 (13.56)
121	Ferulic acid	0.41 (26.56)	0.16 (67.02)	0.15 (101.33)	0.20 (48.01)
122	Dimethoxycinnamic acid	0.04 (15.80)	0.02 (14.99)	0.07 (35.19)	0.02 (16.69)

[a] Colour coding indicates class of compound: carbohydrate-derived, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] Data shown are peak areas as percentages of the total significant compound peak area. Data in brackets are Relative Standard Deviations.

Appendix D

Table 3.5. Py-GC-MS peak area data for depth profile peat samples.

Peak no. [a]	Compound	Orkney profile % peak areas [b]					Glenmachrie profile % peak areas [b]			
		Hp0	Hp1	Hp2	Hp3	Hp4	Lp0	Lp1	Lp2	Lp3
1	2-Methylfuran	1.48	1.10	0.91	0.85	0.76	0.97	0.76	0.81	0.71
2	Acetic acid	7.01	4.40	4.52	4.07	3.58	6.73	3.91	3.70	3.14
3	*trans*-2-Butenal	0.27	0.27	0.26	0.22	0.18	0.29	0.21	0.21	0.15
4	3-Methylbutanal	0.03	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.02
5	Benzene	0.69	0.55	1.39	1.20	1.65	0.86	1.41	1.49	0.98
6	2,3-Pentanedione	0.06	0.03	0.02	0.02	0.02	0.04	0.02	0.03	0.02
7	2-Ethylfuran	0.16	0.12	0.09	0.09	0.09	0.09	0.08	0.09	0.08
8	2,5-Dimethylfuran	0.44	0.36	0.28	0.27	0.25	0.30	0.24	0.25	0.23
9	2,3-Dimethylfuran	0.20	0.13	0.16	0.13	0.12	0.12	0.12	0.13	0.12
10	Vinylfuran	0.29	0.25	0.17	0.15	0.14	0.19	0.14	0.15	0.11
11	1-Methyl-1H-pyrrole	0.20	0.16	0.21	0.25	0.21	0.24	0.28	0.26	0.20
12	Pyridine	0.26	0.17	0.82	0.76	0.93	0.58	0.97	0.90	0.63
13	Pyrrole	0.53	0.49	1.02	0.84	0.91	0.81	1.12	0.90	0.72
14	Toluene	2.18	1.71	3.18	2.69	3.46	2.34	3.24	3.30	2.45
15	(2H)-Furan-3-one	6.34	4.86	3.39	3.04	3.02	4.25	2.83	2.61	2.12
16	3-Furaldehyde	0.82	0.92	0.67	0.48	0.40	0.76	0.54	0.43	0.32
17	2-Methylpyridine	0.00	0.00	0.09	0.09	0.10	0.07	0.10	0.10	0.08
18	Furfural	8.59	7.31	4.57	3.87	3.02	6.48	3.52	3.09	2.61
19	1-Cyclopentene-3,4-dione	0.31	0.49	0.49	0.40	0.34	0.55	0.43	0.34	0.29
20	Ethylbenzene	0.26	0.22	0.43	0.38	0.52	0.28	0.44	0.48	0.35
21	Methylpyridine 2	0.00	0.00	0.04	0.07	0.10	0.03	0.14	0.13	0.08

Peak no. [a]	Compound	Orkney profile % peak areas [b]					Glenmachrie profile % peak areas [b]			
		Hp0	Hp1	Hp2	Hp3	Hp4	Lp0	Lp1	Lp2	Lp3
22	Acetol acetate	0.07	0.05	0.04	0.04	0.03	0.07	0.03	0.03	0.03
23	5-Methyl-2(3H)-furanone	0.45	0.38	0.34	0.31	0.28	0.35	0.25	0.26	0.21
25	Cyclopentenedione 2	0.37	0.42	0.35	0.31	0.30	0.37	0.28	0.27	0.19
26	Cyclopentenedione 3	0.33	0.30	0.35	0.27	0.26	0.31	0.29	0.27	0.20
27	Styrene	0.41	0.36	0.85	0.62	0.91	0.49	0.99	0.75	0.56
28	Xylene 2	0.19	0.16	0.37	0.34	0.47	0.21	0.36	0.41	0.30
29	2-Methyl-2-cyclopentene-1-one	0.34	0.28	0.32	0.29	0.32	0.27	0.27	0.29	0.25
30	(5H)-Furan-2-one	1.84	1.45	1.12	1.03	0.73	1.31	0.85	0.76	0.82
31	2-Acetylfuran	0.19	0.32	0.16	0.11	0.10	0.16	0.12	0.11	0.07
32	Methoxybenzene	0.01	0.02	0.05	0.04	0.06	0.09	0.10	0.14	0.07
33	2-Hydroxy-2-cyclopenten-1-one	2.17	2.05	2.14	1.74	1.52	1.97	1.59	1.40	1.15
34	5-Methyl-2(5H)-furanone	0.41	0.36	0.26	0.31	0.23	0.29	0.31	0.32	0.32
35	3-Methyl-2,5-furandione	0.90	0.60	0.56	0.51	0.44	0.57	0.49	0.48	0.43
36	5-Methylfurfural	2.72	3.77	1.67	1.42	1.26	1.77	1.20	1.26	0.99
37	2H-Pyran-2-one	0.23	0.18	0.19	0.19	0.15	0.20	0.14	0.15	0.11
38	3-Methyl-2(5H)-furanone	0.38	0.29	0.23	0.21	0.22	0.23	0.22	0.16	0.13
39	Phenol	3.37	7.81	12.70	8.04	11.10	6.54	10.44	12.37	11.55
41	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	5.03	6.31	3.62	3.13	1.95	6.79	3.32	2.28	2.49
42	3-Hydroxy-2-methyl-2-cyclopentene-1-one	2.55	2.85	1.96	1.34	1.02	2.09	1.50	1.30	1.03
43	Cyclotene	0.96	1.03	0.80	0.69	0.62	0.87	0.52	0.61	0.43
44	2-Acetyl-5-methylfuran	0.15	0.13	0.13	0.11	0.10	0.10	0.10	0.11	0.09
45	2,3-Dimethyl-2-cyclopenten-1-one	0.18	0.16	0.19	0.19	0.18	0.14	0.17	0.19	0.16
46	4-Methyl-5H-furan-2-one	0.64	0.48	0.43	0.41	0.34	0.40	0.29	0.32	0.29
47	Indene	0.15	0.13	0.31	0.27	0.38	0.17	0.29	0.30	0.21

Peak no. [a]	Compound	Orkney profile % peak areas [b]					Glenmachrie profile % peak areas [b]			
		Hp0	Hp1	Hp2	Hp3	Hp4	Lp0	Lp1	Lp2	Lp3
49	2-Pyridinecarbonitrile	0.03	0.02	0.07	0.04	0.04	0.05	0.06	0.06	0.03
50	Acetophenone	0.16	0.13	0.30	0.26	0.31	0.21	0.31	0.30	0.20
51	*m-/p*-Cresol	2.49	3.37	4.27	3.93	4.77	2.94	4.48	4.65	5.03
52	Methylfuroate	1.45	1.28	0.82	0.50	0.72	0.91	0.68	0.48	0.49
53	Guaiacol	2.39	2.28	2.83	3.76	3.96	2.59	3.49	3.69	3.94
54	Maltol	2.01	1.79	1.65	1.23	1.09	1.63	1.34	1.27	1.14
55	2-methyl-5-hydroxy-(4H)-pyran-4-one	0.50	0.58	0.84	0.61	0.59	0.66	0.55	0.42	0.30
56	2-Ethylphenol	0.06	0.08	0.11	0.09	0.13	0.07	0.10	0.13	0.10
57	Benzyl nitrile	0.20	0.14	0.24	0.17	0.19	0.24	0.26	0.24	0.16
58	Dimethylphenol 1	0.20	0.22	0.30	0.30	0.36	0.20	0.33	0.34	0.37
59	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	0.54	0.40	0.27	0.23	0.19	0.32	0.24	0.19	0.20
60	Dimethylphenol 2	0.09	0.07	0.13	0.10	0.15	0.08	0.14	0.14	0.13
61	3-Methyl-1H-indene	0.05	0.04	0.09	0.08	0.11	0.05	0.07	0.08	0.06
62	4-Ethyl-1,2-dimethylbenzene	0.05	0.04	0.11	0.09	0.09	0.07	0.10	0.13	0.08
63	4-Ethylphenol	0.85	1.24	1.01	1.39	1.59	0.93	1.17	1.48	1.47
65	4-Methoxy-3-methylphenol	0.10	0.11	0.12	0.15	0.15	0.10	0.16	0.14	0.19
66	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	3.01	2.13	1.77	1.51	0.98	1.91	1.27	1.10	1.23
67	Methylguaiacol	2.03	1.77	1.65	2.56	2.24	1.61	2.21	2.29	3.21
70	Dihydrobenzofuran 2	3.89	5.68	5.70	7.68	8.15	6.98	7.08	7.63	7.46
71	Unknown phenolic 1	0.25	0.07	0.20	0.31	0.29	0.16	0.24	0.35	0.31
72	5-Hydroxymethylfurfural	4.56	3.98	1.57	1.29	0.91	2.08	0.87	0.81	0.86
73	Ethylmethylphenol	0.07	0.11	0.14	0.15	0.18	0.10	0.14	0.17	0.17
75	Propenylphenol 1	0.02	0.05	0.06	0.09	0.08	0.06	0.07	0.08	0.08
76	4-Propylphenol	0.02	0.02	0.04	0.05	0.05	0.02	0.04	0.05	0.05

Peak no. [a]	Compound	Orkney profile % peak areas [b]					Glenmachrie profile % peak areas [b]			
		Hp0	Hp1	Hp2	Hp3	Hp4	Lp0	Lp1	Lp2	Lp3
77	Methylbenzenediol	0.56	0.49	0.74	0.85	0.95	0.51	0.68	0.96	0.98
78	3-Methoxy-1,2-benzenediol	0.58	0.65	0.80	1.05	1.08	0.70	0.95	1.15	1.26
79	Ethylguaiacol	0.94	0.90	0.90	1.48	1.37	0.86	1.15	1.36	1.85
80	Indole	0.20	0.18	0.34	0.30	0.42	0.25	0.60	0.33	0.27
81	2-Methyl-2,3-dihydrobenzofuran	0.00	0.71	0.11	0.10	0.09	0.02	0.12	0.09	0.10
83	Vinylguaiacol	4.29	4.80	5.46	7.47	6.98	6.38	6.66	6.80	7.85
84	1,3-Dimethoxybenzene	0.13	0.05	0.16	0.22	0.23	0.12	0.23	0.23	0.26
86	Propenylphenol 2	0.10	0.19	0.19	0.33	0.29	0.21	0.24	0.25	0.27
87	Syringol	0.72	0.74	0.84	1.07	0.98	0.76	0.98	1.05	1.49
88	Eugenol	0.32	0.21	0.23	0.28	0.19	0.25	0.27	0.26	0.39
90	Propylguaiacol	0.20	0.13	0.15	0.19	0.15	0.14	0.19	0.18	0.25
91	4-Ethylcatechol	0.46	0.47	0.81	1.09	1.40	0.50	0.68	1.07	1.10
92	3-Methyl-1H-indole	0.22	0.18	0.33	0.29	0.38	0.23	0.40	0.37	0.29
93	Vanillin	1.30	1.24	1.44	1.76	1.61	1.83	1.90	1.57	1.51
94	*cis*-Isoeugenol	0.25	0.17	0.18	0.23	0.18	0.18	0.21	0.22	0.32
95	4-Acetylphenol	0.31	0.61	0.46	0.54	0.81	0.36	0.48	0.52	0.38
96	Methylsyringol	0.71	0.62	0.66	0.96	0.82	0.57	0.82	0.93	1.54
97	*trans*-Isoeugenol	1.46	0.95	1.02	1.30	0.87	1.07	1.20	1.16	1.77
98	Unknown guaiacyl isomer 1	0.23	0.13	0.18	0.26	0.21	0.21	0.31	0.28	0.25
99	Acetovanillone	1.91	2.05	2.27	2.89	3.03	2.61	3.19	2.95	2.45
100	Unknown guaiacyl isomer 2	0.13	0.08	0.11	0.16	0.13	0.12	0.19	0.17	0.17
101	Unknown phenolic 2	0.09	0.08	0.11	0.15	0.15	0.09	0.20	0.12	0.17
102	Methylvanillate	0.11	0.14	0.16	0.23	0.27	0.17	0.26	0.27	0.18
103	Ethylsyringol	0.18	0.18	0.18	0.27	0.23	0.13	0.21	0.28	0.47
104	Guaiacylacetone	0.65	0.66	0.65	0.92	0.71	0.64	0.83	0.83	0.99

Peak no. [a]	Compound	Orkney profile % peak areas [b]					Glenmachrie profile % peak areas [b]			
		Hp0	Hp1	Hp2	Hp3	Hp4	Lp0	Lp1	Lp2	Lp3
106	Vinylsyringol	1.09	0.79	1.13	1.55	1.36	1.02	1.61	1.37	2.09
107	Vanillic acid	0.48	0.47	0.38	0.61	0.63	0.66	0.43	0.49	0.28
108	Guaiacyl propanaldehyde	0.10	0.09	0.11	0.15	0.14	0.10	0.15	0.15	0.15
109	Propylsyringol	0.05	0.04	0.04	0.08	0.08	0.04	0.06	0.08	0.13
110	Methoxyeugenol	0.12	0.12	0.17	0.25	0.22	0.16	0.21	0.22	0.36
111	Homovanillic acid	0.31	0.11	0.27	0.25	0.11	0.16	0.22	0.19	0.27
112	Syringaldehyde	0.19	0.24	0.29	0.29	0.25	0.23	0.44	0.24	0.35
113	Unknown syringyl monomer 1	0.15	0.10	0.18	0.29	0.24	0.17	0.36	0.26	0.32
114	Unknown syringyl monomer 2	0.07	0.04	0.07	0.13	0.10	0.07	0.17	0.09	0.13
115	Propenylsyringol	0.83	0.70	0.93	1.39	1.21	1.02	1.15	1.15	1.92
116	Acetosyringone	0.71	0.83	0.99	0.97	0.92	0.88	1.15	1.12	1.31
118	Syringylpropanone	0.37	0.55	0.55	0.86	0.77	0.47	0.71	0.73	1.05
119	Syringic acid	0.16	0.06	0.06	0.10	0.07	0.14	0.06	0.09	0.06
120	Syringyl propanaldehyde	0.05	0.06	0.07	0.11	0.11	0.07	0.09	0.09	0.12
121	Ferulic acid	0.02	0.12	0.11	0.13	0.12	0.10	0.10	0.10	0.13
122	Dimethoxycinnamic acid	0.02	0.03	0.03	0.03	0.03	0.02	0.04	0.04	0.05

[a] Colour coding indicates class of compound: carbohydrate-derived, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] Data shown are percentages of the total peak area.

Appendix E

Table 3.9. Compounds detected in peated malt using SPE and HS-SPME in conjunction with GC-MS.

Peak no. [a,b]	Compound	RT [c]	Estimated RI	Literature RI [d]	QI	MW
1	Pyridine	11.62	1145	**1192**	79	79
2	2-Methylcyclopentanone	11.94	1156		42, 98	98
3	2-Methylpyridine	12.67	1182	1221	93	93
4	2,6-Lutidine	13.61	1215	1276	106, 107	107
5	2-Methylpyrazine	14.28	1238	1267	94	94
6	2-Ethylpyridine	14.77	1256	1282	106, 107	107
7	Ethylpicoline 1	15.19	1271		120, 121	121
8	Picoline 2	15.38	1277		93	93
9	Picoline 3	15.63	1286		93	93
10	Lutidine 2	16.34	1311		106, 107	107
11	Lutidine 3	16.72	1325		106, 107	107
12	Lutidine 4	17.51	1352		106, 107	107
13	2-Cyclopenten-1-one	17.52	1353		82	82
14	Trimethylpyridine 1	17.64	1357		121	121
15	2-Methyl-2-cyclopenten-1-one	17.87	1365	1367	67, 96	96
16	Trimethylpyridine 2	18.17	1376		121	121
17	3-Ethylpyridine	18.29	1380	1387	92, 107	107
18	Ethylpicoline 2	18.45	1386		120, 121	121
19	4-Ethylpyridine	18.69	1394	1380	106, 107	107
20	Ethylpicoline 3	18.70	1394		120, 121	121
21	Ethylpicoline 4	19.00	1405		120, 121	121
22	Ethylpicoline 5	19.19	1412		106, 121	121
23	Lutidine 5	19.44	1421		106, 107	107
24	Ethyllutidine	19.74	1431		134, 135	135
25	Acetic acid	20.00	1440	**1447**	60	60
26	Dimethylcyclopentenone 1	20.40	1454		67, 110	110
27	Furfural	20.81	1469	**1468**	96	96
28	Ethenylpyridine	21.35	1488		104, 105	105
29	Trimethylpyridine 3	21.35	1488		120, 121	121
30	Lutidine 6	21.43	1491		106, 107	107
31	Dimethylcyclopentenone 2	21.64	1498		95, 110	110
32	Indene	21.84	1505		115, 116	116
33	Trimethylcyclopentenone 1	22.01	1511		109, 124	124
34	Benzofuran	22.19	1518		89, 90, 118	118
35	2-Acetylfuran	22.25	1520	1487	95, 110	110
36	Trimethylpyridine 4	22.34	1523		120, 121	121
37	3-Methyl-2-cyclopenten-1-one	22.97	1545	1535	67, 96	96
38	2,3-Dimethylcyclopentene-1-one	23.54	1565		67, 110	110
39	3,4-Dimethylcyclopentene-1-one	23.64	1569		95, 110	110
40	Trimethylcyclopentenone 2	23.94	1579		109, 124	124

Appendix E

Peak no. [a,b]	Compound	RT [c]	Estimated RI	Literature RI [d]	QI	MW
41	Dihydromethylenefuranone	24.25	1590		68, 98	98
42	5-Methylfurfural	24.32	1593	**1568**	53, 110	110
43	3-Methoxypyridine	24.69	1606		66, 109	109
44	2-Methylbenzofuran	25.06	1619		131, 132	132
45	2-Acetyl-1-methylpyrrole	25.40	1631	1645	108, 123	123
46	Benzonitrile	25.53	1635	1591	103	103
47	2-Acetyl-5-methylfuran	25.63	1639	1848	109, 124	124
48	2,3-Dihydro-1H-indole	25.90	1649		118, 119	119
49	3-Ethyl-2-cyclopenten-1-one	26.38	1665		81, 110	110
50	Furfuryl alcohol	26.51	1670	**1665**	98	98
51	Benzeneacetaldehyde	26.58	1673	1642	91, 120	120
52	Acetophenone	26.86	1682	**1653**	77, 105	120
53	2-Methylbenzoxazole	27.66	1711		133	133
54	5-Methyl-2-furanmethanol	28.30	1733		95, 112	112
55	3-Hydroxybenzonitrile	28.70	1747		119	119
56	Methylfuranone	28.99	1758		69, 98	98
57	Naphthalene	29.67	1782	**1735**	128	128
58	Pyridinecarbonitrile 1	29.76	1785		77, 104	104
59	Methylacetophenone 1	29.90	1790		119, 134	134
60	Methylacetophenone 2	30.56	1813		119, 134	134
61	Dimethoxytoluene 1	30.88	1824		137, 152	152
62	1-Phenylethanol	31.11	1832		79, 107	122
63	4-Acetylphenol	31.36	1841		121, 136	136
64	Cyclotene	31.70	1853	1829	69, 112	112
65	1-(3-Pyridinyl)-ethanone	32.11	1868		78, 106	121
66	2-Chlorophenol	32.15	1869		128	128
67	2-Methylnaphthalene	32.85	1894	1876	141, 142	142
68	Benzyl alcohol	32.93	1897	1881	79, 108	108
69	2,6-Dimethylphenol	33.76	1926	1889	107, 122	122
70	1-Methylnaphthalene	33.85	1929	1915	141, 142	142
71	Pyridinecarbontrile 2	34.20	1941		77, 104	104
72	Hydroxymethylacetophenone	34.41	1949		135, 150	150
73	Benzylnitrile	34.65	1957	1894	90, 117	117
74	Methoxymethylphenol	34.79	1962		123, 138	138
75	Quinoline	35.28	1980		129	129
76	Dimethoxytoluene 2	35.38	1983		137, 152	152
77	Maltol	35.56	1989		126	126
78	2-Acetylpyrrole	35.62	1992	1973	94, 109	109
79	Methylquinoline 1	35.95	2003		143	143
80	Biphenyl	36.33	2017		154	154
81	Isoquinoline	36.46	2021		129	129
82	Methylindanone 1	36.78	2032		131, 146	146
83	Methylindanone 2	37.54	2059		117, 146	146
84	2-Ethylphenol	37.76	2067		107, 122	122
85	3-Hydroxy-2,6-dimethyl-4H-pyran-4-one	37.88	2071		140	140
86	Dimethylphenol 2 + 3	38.07	2078		122	122

Peak no. [a,b]	Compound	RT [c]	Estimated RI	Literature RI [d]	QI	MW
87	2,4-Dimethoxyphenol	38.60	2097		139, 154	154
88	Dimethylquinoline	38.92	2108		157	157
89	Propylguaiacol	39.01	2111	**2138**	137, 166	166
90	5-Methyl-1H-pyrrole-2-carboxaldehyde	39.04	2112		108, 109	109
91	C3 phenol 1	39.41	2125		121	136
92	C3 phenol 2	39.56	2131		121	136
93	Dimethylphenol 4	39.78	2138		107, 122	122
94	Methylquinoline 2	39.83	2140		143	143
95	Methylindanone 3	40.03	2147		117, 146	146
96	C3 phenol 3	40.12	2150		121, 136	136
97	C3 phenol 4	40.23	2154		121, 136	136
98	Eugenol	40.42	2161	**2169**	149, 164	164
99	3-Ethylphenol	40.61	2168	2221	107, 122	122
100	Methylindanone 4	40.97	2180		117, 146	146
101	Vinylguaiacol	41.16	2187	**2186**	135, 150	150
102	C3 phenol 5	41.25	2190	2178	121, 136	136
103	C3 phenol 6	41.47	2198		121, 136	136
104	C3 phenol 7	42.37	2230		121, 136	136
105	4-Propylphenol	42.51	2235		107, 136	136
106	Propenylmethoxyphenol 1	42.56	2236		149, 164	164
107	Syringol	42.73	2242	2302	139, 154	154
108	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	43.26	2261		142	142
109	Dibenzofuran	43.40	2266		139, 168	168
110	Propenylmethoxyphenol 2	44.07	2290		149, 164	164
111	Propenylphenol 1	44.22	2295	2340	133, 134	134
112	1-Indolinecarboxaldehyde	44.50	2305		118, 147	147
113	Methylsyringol	44.65	2310		153, 168	168
114	Propenylmethoxyphenol 3	44.67	2311		164	164
115	2,3-Dihydrobenzofuran	44.87	2318		91, 120	120
116	4-Butylphenol	44.99	2322		107, 150	150
117	Propenylphenol 2	45.08	2325	2340	134	134
118	Dihydrobenzofuran 2	45.47	2339	2401	91, 120	120
119	1(3h)-Isobenzofuranone	45.63	2345		105, 134	134
120	Ethylsyringol	45.88	2354		167, 182	182
121	Pyridinol	46.20	2365		95	95
122	Benzoic acid	46.32	2369	**2448**	105, 122	122
123	3-Methoxyphenol	46.58	2378		124	124
124	2-Methyl-2,3-dihydrobenzofuran	46.75	2384		119, 134	134
125	2,3-Dihydro-1H-inden-5-ol	46.88	2389		133, 134	134
126	Indole	47.01	2393	**2424**	90, 117	117
127	Propylsyringol	47.38	2407		167, 196	196
128	5-(Hydroxymethyl)-2-furaldehyde	47.97	2427		97, 126	126
129	3-Methyl-1H-indole	48.03	2429		130, 131	131
130	4-Propenylsyringol	48.70	2453	2533	194	194

Peak no. [a,b]	Compound	RT [c]	Estimated RI	Literature RI [d]	QI	MW
131	Benzeneacetic acid	48.97	2463	2575	91, 136	136
132	Vanillin	49.67	2487	2569	151, 152	152
133	Vanillic acid methyl ester	50.43	2514	2607	151, 182	182
134	Acetovanillone	51.48	2551	2663	151, 166	166
135	Guaiacyl acetone	51.88	2565		137, 180	180
136	Pyrocatechol	52.91	2602		110	110
137	Guaiacyl propanaldehyde	53.12	2609		151, 180	180
138	Unknown phenolic	55.15	2681		137, 210	?
139	Guaiacol	32.57	1884	**1868**	109, 124	124
140	Methylguaiacol	35.15	1975	**1968**	123, 138	138
141	*o*-Cresol	36.11	2009	**2026**	108	108
142	Phenol	36.23	2013	**1994**	94	94
143	Ethylguaiacol	37.03	2041	**2034**	137, 152	152
144	*p*-Cresol	38.17	2081	**2079**	108	108
145	*m*-Cresol	38.37	2089	**2095**	108	108
146	4-Ethylphenol	40.43	2161	**2095**	107, 122	122

[a] Colour coding indicates class of compound: carbohydrate-derived, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] Peaks 1 to 138 were quantified using SPE and 139 to 146 using HS-SPME.

[c] All RT values are for SPE analysis.

[d] Literature RI data taken from [153,154,158-162]. Estimated RI were calculated as described in Chapter 2.4.2 using literature RI data for selected compounds (identified in bold).

Appendix F

Table 3.10. Mean response ratios (analyte peak areas relative to internal standard peak areas) of peat-derived compounds measured in peated and unpeated lab-scale malts (for each malt n=3).

Peak no. [a]	Compound	Gar [b]	Ork [b]	Gle [b]	St F [b]	Cas [b]	Tom [b]	Unpeat [b]	p value [c]
1	Pyridine	3.17 (12.30)	2.46 (16.87)	3.23 (6.35)	2.84 (10.22)	3.23 (10.30)	2.52 (20.18)	0.00 (0.00)	0.0679
2	2-Methylcyclopentanone	0.03 (26.02)	0.02 (41.13)	0.02 (18.36)	0.02 (27.69)	0.03 (10.64)	0.02 (33.22)	0.00 (0.00)	0.0153
3	2-Methylpyridine	2.73 (9.17)	1.78 (30.16)	2.68 (12.61)	2.16 (11.99)	2.99 (7.58)	1.70 (16.68)	0.01 (87.13)	0.0017
4	2,6-Lutidine	1.14 (5.87)	0.69 (36.03)	1.12 (21.47)	0.83 (13.43)	1.26 (13.30)	0.56 (20.33)	0.00 (0.00)	0.0016
5	2-Methylpyrazine	0.69 (7.73)	0.42 (19.30)	0.65 (9.89)	0.62 (12.19)	0.73 (16.34)	0.48 (23.98)	0.00 (0.00)	0.0056
6	2-Ethylpyridine	2.21 (13.22)	1.46 (29.26)	2.09 (11.27)	1.60 (10.26)	2.52 (8.46)	1.14 (12.59)	0.00 (0.00)	0.0003
7	Ethylpicoline 1	1.49 (9.87)	0.94 (35.29)	1.44 (15.94)	0.95 (10.49)	1.64 (8.58)	0.64 (14.62)	0.00 (0.00)	0.0002
8	Picoline 2	4.18 (12.48)	2.86 (24.92)	3.98 (9.56)	3.66 (11.06)	4.48 (10.58)	2.88 (17.70)	0.01 (89.94)	0.0087
9	Picoline 3	1.58 (8.07)	1.01 (32.56)	1.54 (12.74)	1.35 (12.80)	1.80 (11.19)	0.95 (20.06)	0.00 (0.00)	0.0019
10	Lutidine 2	2.88 (5.75)	1.79 (36.93)	2.79 (18.94)	2.22 (13.70)	3.41 (10.04)	1.50 (16.16)	0.00 (173.21)	0.0008
11	Lutidine 3	2.21 (4.84)	1.32 (32.75)	2.16 (20.04)	1.77 (13.01)	2.71 (12.15)	1.12 (20.93)	0.04 (92.41)	0.0004
12	Lutidine 4	1.78 (8.93)	1.04 (32.73)	1.65 (17.67)	1.35 (10.01)	2.15 (12.04)	0.79 (20.85)	0.00 (0.00)	0.0002
13	2-Cyclopenten-1-one	0.02 (2.90)	0.02 (14.57)	0.01 (5.78)	0.02 (19.30)	0.01 (5.72)	0.03 (8.01)	0.00 (32.44)	0.0000
14	Trimethylpyridine 1	0.25 (8.09)	0.15 (32.84)	0.23 (29.72)	0.19 (16.48)	0.29 (20.66)	0.11 (24.15)	0.00 (0.00)	0.0033
15	2-Methyl-2-cyclopenten-1-one	0.12 (5.69)	0.11 (25.48)	0.11 (6.71)	0.12 (12.64)	0.12 (11.32)	0.14 (9.34)	0.00 (22.78)	0.1125
16	Trimethylpyridine 2	0.77 (7.61)	0.43 (22.87)	0.71 (8.48)	0.55 (8.96)	0.92 (11.45)	0.42 (22.36)	0.01 (91.91)	0.0000
17	3-Ethylpyridine	2.85 (14.67)	1.76 (17.69)	2.73 (7.72)	2.00 (12.11)	2.90 (16.17)	1.89 (22.45)	0.00 (0.00)	0.0041
18	Ethylpicoline 2	2.41 (11.85)	1.40 (22.40)	2.37 (9.03)	1.54 (12.21)	2.68 (15.56)	1.24 (18.41)	0.00 (0.00)	0.0001
19	4-Ethylpyridine	1.47 (8.80)	0.86 (29.02)	1.35 (13.56)	1.16 (12.25)	1.80 (11.19)	0.77 (23.25)	0.00 (0.00)	0.0002
20	Ethylpicoline 3	1.81 (8.71)	1.10 (34.67)	1.74 (19.84)	1.32 (9.44)	2.32 (11.13)	0.81 (18.73)	0.00 (0.00)	0.0001
21	Ethylpicoline 4	0.85 (8.61)	0.52 (31.77)	0.77 (15.57)	0.57 (10.15)	0.98 (12.25)	0.37 (15.95)	0.00 (0.00)	0.0001
22	Ethylpicoline 5	1.75 (7.17)	1.02 (26.20)	1.67 (14.12)	1.13 (10.89)	2.03 (16.19)	0.86 (16.85)	0.00 (0.00)	0.0001
23	Lutidine 5	2.11 (8.02)	1.35 (29.91)	1.96 (15.95)	1.75 (10.98)	2.42 (12.34)	1.25 (20.89)	0.00 (0.00)	0.0021
24	Ethyllutidine	0.70 (6.86)	0.35 (25.28)	0.65 (15.41)	0.49 (9.02)	0.89 (10.25)	0.25 (17.03)	0.00 (0.00)	0.0000
25	Acetic acid	0.04 (26.25)	0.04 (12.27)	0.05 (9.06)	0.03 (12.99)	0.02 (54.74)	0.04 (36.55)	0.01 (63.58)	0.0335
26	Dimethylcyclopentenone 1	0.02 (15.32)	0.02 (19.78)	0.02 (8.64)	0.02 (9.76)	0.02 (10.79)	0.02 (0.45)	0.00 (86.74)	0.9099
27	Furfural	0.10 (36.92)	0.13 (9.23)	0.12 (30.28)	0.11 (49.50)	0.06 (49.66)	0.23 (26.92)	0.00 (92.17)	0.0091
28	Ethenylpyridine	0.93 (19.36)	0.58 (18.74)	0.90 (10.05)	0.61 (13.74)	0.94 (14.57)	0.63 (27.75)	0.00 (0.00)	0.0107
29	Trimethylpyridine 3	0.37 (4.50)	0.25 (27.16)	0.41 (45.80)	0.31 (10.93)	0.50 (19.96)	0.19 (41.54)	0.00 (0.00)	0.0227

Peak no. [a]	Compound	Gar [b]	Ork [b]	Gle [b]	St F [b]	Cas [b]	Tom [b]	Unpeat [b]	p value [c]
30	Lutidine 6	1.14 (3.40)	0.64 (36.15)	0.98 (19.40)	0.99 (12.70)	1.84 (22.71)	0.50 (29.72)	0.00 (0.00)	0.0002
31	Dimethylcyclopentenone 2	0.04 (6.83)	0.04 (21.01)	0.04 (5.89)	0.04 (14.64)	0.04 (11.65)	0.05 (5.40)	0.00 (0.00)	0.7182
32	Indene	0.24 (18.20)	0.27 (15.76)	0.21 (10.96)	0.25 (11.32)	0.22 (19.86)	0.22 (8.82)	0.00 (0.00)	0.3450
33	Trimethylcyclopentenone 1	0.02 (16.86)	0.02 (20.68)	0.02 (5.93)	0.02 (11.90)	0.02 (11.79)	0.02 (0.50)	0.00 (0.00)	0.7941
34	Benzofuran	0.22 (17.97)	0.25 (15.48)	0.22 (5.62)	0.29 (9.44)	0.19 (16.95)	0.29 (6.78)	0.00 (0.00)	0.0077
35	2-Acetylfuran	0.21 (6.66)	0.25 (14.93)	0.22 (2.99)	0.19 (12.62)	0.18 (5.92)	0.38 (3.19)	0.00 (9.47)	0.0000
36	Trimethylpyridine 4	0.44 (5.26)	0.25 (35.19)	0.41 (29.26)	0.35 (10.67)	0.60 (14.74)	0.19 (29.26)	0.00 (0.00)	0.0004
37	3-Methyl-2-cyclopenten-1-one	0.11 (6.55)	0.11 (18.15)	0.10 (2.51)	0.10 (13.96)	0.10 (7.97)	0.12 (4.69)	0.00 (15.79)	0.4378
38	2,3-Dimethylcyclopentene-1-one	0.24 (4.71)	0.24 (24.16)	0.24 (4.47)	0.24 (14.96)	0.24 (9.37)	0.27 (7.93)	0.00 (15.48)	0.8003
39	3,4-Dimethylcyclopentene-1-one	0.02 (1.52)	0.02 (24.28)	0.02 (6.12)	0.02 (11.09)	0.02 (11.26)	0.02 (4.94)	0.00 (0.00)	0.8887
40	Trimethylcyclopentenone 2	0.02 (12.42)	0.02 (18.72)	0.02 (4.21)	0.02 (11.34)	0.02 (12.56)	0.02 (3.71)	0.00 (0.00)	0.6892
41	Dihydromethylenefuranone	0.02 (7.09)	0.02 (25.31)	0.02 (9.13)	0.01 (15.20)	0.02 (10.78)	0.02 (15.13)	0.00 (0.00)	0.0115
42	5-Methylfurfural	0.58 (27.78)	0.75 (7.65)	0.58 (20.36)	0.60 (40.59)	0.43 (45.16)	1.53 (23.44)	0.00 (59.13)	0.0004
43	3-Methoxypyridine	20.16 (4.87)	8.43 (22.25)	20.46 (0.99)	14.87 (10.82)	22.62 (17.61)	11.09 (25.93)	0.08 (36.08)	0.0000
44	2-Methylbenzofuran	0.20 (22.91)	0.22 (20.74)	0.22 (16.64)	0.26 (10.69)	0.20 (25.19)	0.26 (8.84)	0.00 (0.00)	0.3230
45	2-Acetyl-1-methylpyrrole	5.29 (7.29)	1.90 (19.89)	5.41 (2.96)	3.06 (11.76)	6.47 (17.17)	2.31 (25.56)	0.00 (0.00)	0.0000
46	Benzonitrile	8.06 (23.41)	6.63 (15.44)	7.85 (16.78)	6.37 (9.45)	7.03 (18.56)	5.23 (27.85)	0.00 (0.00)	0.1775
47	2-Acetyl-5-methylfuran	0.11 (11.11)	0.12 (16.95)	0.11 (3.58)	0.09 (13.96)	0.09 (9.81)	0.16 (7.66)	0.00 (1.37)	0.0002
48	2,3-Dihydro-1H-indole	3.29 (8.11)	2.08 (18.67)	2.94 (4.44)	1.90 (11.38)	3.98 (17.97)	1.46 (27.59)	0.00 (0.00)	0.0000
49	3-Ethyl-2-cyclopenten-1-one	0.08 (3.48)	0.07 (25.05)	0.07 (7.20)	0.08 (12.97)	0.08 (9.64)	0.08 (10.64)	0.00 (7.11)	0.5592
50	Furfuryl alcohol	0.09 (5.61)	0.12 (35.77)	0.10 (13.52)	0.07 (28.09)	0.07 (23.12)	0.15 (17.17)	0.00 (13.97)	0.0082
51	Benzeneacetaldehyde	1.43 (8.04)	1.40 (9.44)	1.80 (4.33)	1.35 (9.57)	1.53 (11.64)	1.54 (7.07)	0.75 (15.75)	0.0118
52	Acetophenone	4.18 (15.83)	4.24 (11.98)	3.93 (7.15)	3.55 (13.46)	3.72 (18.59)	4.24 (11.61)	0.03 (3.37)	0.5126
53	2-Methylbenzoxazole	2.36 (21.58)	1.52 (17.51)	2.47 (13.19)	1.77 (10.66)	2.51 (14.48)	2.13 (26.55)	0.00 (0.00)	0.0472
54	5-Methyl-2-furanmethanol	0.11 (10.63)	0.13 (54.55)	0.13 (6.32)	0.09 (35.43)	0.14 (48.91)	0.23 (26.98)	0.00 (58.27)	0.0558
55	3-Hydroxybenzonitrile	3.22 (10.52)	1.95 (16.33)	3.11 (7.98)	2.52 (10.96)	3.07 (15.75)	3.47 (31.33)	0.00 (0.00)	0.0480
56	Methylfuranone	0.02 (6.53)	0.02 (20.86)	0.02 (4.07)	0.02 (13.04)	0.02 (13.27)	0.04 (13.46)	0.00 (20.53)	0.0000
57	Naphthalene	0.70 (22.12)	0.75 (21.96)	0.66 (12.92)	0.65 (7.06)	0.67 (17.61)	0.61 (4.82)	0.09 (5.75)	0.7115
58	Pyridinecarbonitrile 1	0.85 (7.00)	0.46 (25.06)	0.71 (23.45)	0.74 (12.71)	0.83 (26.09)	0.47 (34.70)	0.00 (0.00)	0.0196
59	Methylacetophenone 1	0.95 (10.46)	0.94 (19.97)	0.89 (7.20)	0.87 (11.59)	0.90 (17.02)	1.06 (8.41)	0.00 (0.00)	0.5109
60	Methylacetophenone 2	0.91 (11.23)	0.84 (15.13)	0.89 (7.18)	0.88 (10.11)	0.89 (18.43)	0.98 (11.08)	0.01 (15.00)	0.7362
61	Dimethoxytoluene 1	0.43 (8.85)	0.22 (19.23)	0.51 (8.54)	0.85 (13.55)	0.63 (16.46)	0.22 (12.93)	0.00 (0.00)	0.0000
62	1-Phenylethanol	0.54 (20.91)	0.52 (43.86)	0.56 (17.49)	0.41 (33.77)	0.69 (32.70)	0.51 (22.23)	0.00 (0.00)	0.4791
63	4-Acetylphenol	0.06 (16.16)	0.06 (16.51)	0.07 (4.84)	0.05 (11.89)	0.06 (15.44)	0.07 (6.65)	0.00 (0.00)	0.1903
64	Cyclotene	0.17 (10.12)	0.16 (24.28)	0.16 (10.54)	0.17 (15.99)	0.21 (15.61)	0.27 (15.16)	0.01 (7.40)	0.0029
65	1-(3-Pyridinyl)-ethanone	1.25 (11.91)	0.74 (19.79)	1.11 (5.38)	0.99 (18.12)	1.23 (19.37)	1.25 (34.19)	0.00 (0.00)	0.1069

Peak no. [a]	Compound	Gar [b]	Ork [b]	Gle [b]	St F [b]	Cas [b]	Tom [b]	Unpeat [b]	p value [c]
66	2-Chlorophenol	0.01 (12.19)	0.01 (14.64)	0.01 (5.06)	0.00 (13.25)	0.01 (12.48)	0.01 (8.23)	0.00 (0.00)	0.0000
67	2-Methylnaphthalene	0.88 (23.29)	0.78 (31.03)	0.83 (13.35)	0.62 (9.20)	0.89 (14.69)	0.65 (1.59)	0.03 (15.35)	0.1894
68	Benzyl alcohol	1.16 (9.19)	1.24 (16.80)	1.21 (11.73)	0.93 (9.89)	1.45 (21.03)	0.95 (1.74)	0.45 (5.69)	0.0252
69	2,6-Dimethylphenol	0.08 (10.28)	0.07 (22.84)	0.09 (8.06)	0.08 (14.28)	0.09 (13.74)	0.07 (7.72)	0.00 (0.00)	0.1773
70	1-Methylnaphthalene	0.62 (27.88)	0.59 (33.18)	0.58 (12.22)	0.43 (8.85)	0.62 (16.32)	0.46 (3.27)	0.02 (4.40)	0.2801
71	Pyridinecarbontrile 2	3.39 (6.39)	1.93 (20.90)	2.76 (6.38)	3.06 (9.48)	3.70 (21.10)	2.16 (30.35)	0.00 (0.00)	0.0042
72	Hydroxymethylacetophenone	0.02 (13.93)	0.02 (19.28)	0.02 (6.84)	0.02 (11.42)	0.02 (14.08)	0.02 (6.29)	0.00 (0.00)	0.0203
73	Benzylnitrile	8.38 (17.80)	4.94 (19.40)	9.01 (10.85)	6.23 (16.78)	9.31 (17.76)	6.22 (37.58)	0.00 (0.00)	0.0179
74	Methoxymethylphenol	0.08 (9.87)	0.04 (19.06)	0.09 (5.50)	0.12 (15.38)	0.10 (16.79)	0.04 (14.91)	0.00 (0.00)	0.0000
75	Quinoline	5.80 (6.40)	4.13 (18.59)	5.09 (2.65)	4.46 (8.65)	5.07 (16.04)	5.66 (32.20)	0.01 (173.21)	0.2421
76	Dimethoxytoluene 2	0.92 (9.53)	0.58 (21.67)	1.19 (11.95)	1.53 (17.07)	1.24 (18.55)	0.83 (13.71)	0.00 (0.00)	0.0003
77	Maltol	0.18 (7.95)	0.18 (22.17)	0.18 (12.88)	0.19 (15.77)	0.17 (16.51)	0.32 (17.96)	0.00 (75.81)	0.0016
78	2-Acetylpyrrole	18.92 (10.71)	10.67 (23.93)	18.72 (2.75)	13.08 (10.14)	22.29 (19.55)	17.30 (31.91)	0.09 (19.90)	0.0090
79	Methylquinoline 1	2.62 (9.98)	1.78 (20.72)	2.47 (3.93)	1.76 (8.25)	2.67 (15.59)	2.04 (27.50)	0.00 (0.00)	0.0183
80	Biphenyl	0.42 (24.79)	0.41 (16.69)	0.43 (7.97)	0.35 (11.70)	0.42 (14.57)	0.45 (11.42)	0.00 (0.00)	0.5334
81	Isoquinoline	3.24 (9.45)	2.15 (22.04)	2.85 (1.39)	2.67 (7.43)	3.42 (17.98)	2.74 (33.25)	0.00 (0.00)	0.1092
82	Methylindanone 1	0.72 (3.27)	0.68 (21.81)	0.72 (3.45)	0.63 (10.06)	0.83 (14.82)	0.77 (14.96)	0.00 (0.00)	0.2361
83	Methylindanone 2	1.04 (1.02)	1.01 (20.83)	0.96 (5.43)	1.08 (12.96)	1.05 (14.98)	1.35 (15.02)	0.00 (0.00)	0.0887
84	2-Ethylphenol	0.24 (6.15)	0.17 (20.83)	0.23 (9.34)	0.20 (12.13)	0.25 (15.14)	0.16 (12.49)	0.00 (87.73)	0.0082
85	3-Hydroxy-2,6-dimethyl-4H-pyran-4-one	0.02 (8.17)	0.02 (25.07)	0.02 (10.02)	0.01 (11.22)	0.02 (11.25)	0.02 (16.45)	0.00 (0.00)	0.0156
86	Dimethylphenol 2 + 3	0.46 (5.84)	0.32 (22.05)	0.44 (10.90)	0.48 (16.19)	0.49 (11.70)	0.42 (12.78)	0.00 (0.00)	0.0318
87	2,4-Dimethoxyphenol	0.02 (1.99)	0.02 (14.13)	0.02 (8.16)	0.03 (10.42)	0.03 (11.87)	0.01 (37.65)	0.00 (34.94)	0.0002
88	Dimethylquinoline	0.98 (6.52)	0.64 (20.15)	0.92 (5.75)	0.65 (9.81)	1.03 (14.21)	0.72 (32.86)	0.00 (0.00)	0.0093
89	Propylguaiacol	0.10 (12.51)	0.06 (16.87)	0.16 (9.46)	0.17 (14.52)	0.14 (18.25)	0.08 (18.54)	0.00 (0.00)	0.0000
90	5-Methyl-1H-pyrrole-2-carboxaldehyde	5.63 (7.36)	3.14 (22.20)	5.75 (5.59)	4.26 (11.68)	7.42 (15.69)	5.83 (34.19)	0.00 (0.00)	0.0047
91	C3 phenol 1	0.06 (8.44)	0.04 (19.01)	0.06 (13.71)	0.05 (12.08)	0.07 (16.65)	0.04 (12.00)	0.00 (0.00)	0.0011
92	C3 phenol 2	0.02 (8.01)	0.02 (17.84)	0.02 (13.83)	0.02 (14.09)	0.03 (17.22)	0.02 (12.22)	0.00 (0.00)	0.0411
93	Dimethylphenol 4	0.17 (2.99)	0.14 (20.90)	0.15 (12.09)	0.20 (13.00)	0.17 (13.87)	0.17 (14.91)	0.00 (173.21)	0.0514
94	Methylquinoline 2	2.21 (8.00)	1.44 (18.66)	1.98 (3.25)	1.66 (7.05)	2.09 (15.72)	2.16 (33.48)	0.00 (0.00)	0.1135
95	Methylindanone 3	1.34 (1.65)	1.25 (20.87)	1.22 (5.57)	1.41 (10.65)	1.31 (11.47)	1.76 (15.99)	0.00 (0.00)	0.0350
96	C3 phenol 3	0.27 (6.62)	0.18 (18.44)	0.29 (18.27)	0.26 (13.15)	0.34 (28.07)	0.21 (13.70)	0.00 (87.60)	0.0272
97	C3 phenol 4	0.06 (6.11)	0.04 (20.47)	0.05 (13.15)	0.06 (12.81)	0.07 (12.37)	0.05 (13.87)	0.00 (0.00)	0.0163
98	Eugenol	0.06 (6.66)	0.04 (16.55)	0.07 (8.75)	0.11 (15.42)	0.08 (18.86)	0.05 (39.32)	0.00 (0.00)	0.0003
99	3-Ethylphenol	0.43 (0.74)	0.25 (20.97)	0.33 (11.91)	0.48 (11.76)	0.46 (15.21)	0.32 (15.52)	0.00 (17.66)	0.0007
100	Methylindanone 4	1.35 (3.17)	1.24 (20.29)	1.25 (6.83)	1.46 (9.54)	1.32 (11.89)	2.00 (24.80)	0.00 (0.00)	0.0213

Peak no. [a]	Compound	Gar [b]	Ork [b]	Gle [b]	St F [b]	Cas [b]	Tom [b]	Unpeat [b]	p value [c]
101	Vinylguaiacol	1.20 (6.07)	0.74 (15.52)	1.66 (10.23)	1.43 (25.29)	1.44 (23.12)	0.80 (23.70)	0.00 (50.71)	0.0018
102	C3 phenol 5	0.04 (6.24)	0.04 (19.77)	0.04 (14.49)	0.05 (10.95)	0.04 (16.32)	0.05 (12.74)	0.00 (0.00)	0.0360
103	C3 phenol 6	0.06 (11.91)	0.04 (19.57)	0.06 (12.15)	0.09 (12.46)	0.06 (20.19)	0.15 (29.31)	0.00 (0.00)	0.0002
104	C3 phenol 7	0.10 (3.91)	0.06 (22.03)	0.08 (15.73)	0.10 (12.23)	0.12 (14.80)	0.07 (13.18)	0.00 (0.00)	0.0017
105	4-Propylphenol	0.13 (7.39)	0.07 (21.15)	0.10 (12.79)	0.14 (16.69)	0.13 (20.80)	0.08 (17.68)	0.00 (0.00)	0.0017
106	Propenylmethoxyphenol 1	0.03 (9.50)	0.02 (29.13)	0.05 (10.87)	0.07 (24.91)	0.05 (22.19)	0.02 (30.23)	0.00 (0.00)	0.0001
107	Syringol	0.98 (2.20)	0.45 (22.86)	1.26 (12.45)	1.74 (17.90)	1.40 (18.06)	0.59 (25.56)	0.00 (28.73)	0.0000
108	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	0.03 (9.13)	0.03 (17.64)	0.04 (13.10)	0.03 (21.23)	0.03 (33.97)	0.07 (22.07)	0.00 (0.00)	0.0007
109	Dibenzofuran	0.38 (10.96)	0.34 (24.94)	0.28 (19.79)	0.60 (22.07)	0.28 (7.18)	0.47 (18.26)	0.00 (0.00)	0.0023
110	Propenylmethoxyphenol 2	0.01 (9.79)	0.01 (21.06)	0.02 (11.18)	0.02 (13.57)	0.02 (18.61)	0.01 (20.27)	0.00 (0.00)	0.0001
111	Propenylphenol 1	0.03 (7.30)	0.02 (30.55)	0.03 (16.15)	0.03 (44.87)	0.03 (18.26)	0.04 (15.58)	0.00 (0.00)	0.0618
112	1-Indolinecarboxaldehyde	2.93 (7.47)	1.56 (19.53)	2.83 (4.95)	2.13 (8.39)	3.23 (11.45)	2.02 (32.57)	0.00 (0.00)	0.0007
113	Methylsyringol	0.45 (3.65)	0.23 (23.50)	0.66 (15.42)	1.22 (20.39)	0.73 (20.29)	0.40 (24.34)	0.00 (0.00)	0.0000
114	Propenylmethoxyphenol 3	0.12 (7.01)	0.06 (30.28)	0.16 (6.90)	0.26 (26.53)	0.19 (21.25)	0.08 (28.34)	0.00 (0.00)	0.0002
115	2,3-Dihydrobenzofuran	1.65 (4.49)	0.99 (21.22)	1.31 (10.87)	2.19 (14.66)	1.82 (16.01)	1.84 (24.91)	0.00 (0.00)	0.0028
116	4-Butylphenol	0.03 (20.19)	0.02 (14.01)	0.03 (15.42)	0.08 (8.23)	0.03 (7.45)	0.02 (24.25)	0.00 (0.00)	0.0000
117	Propenylphenol 2	0.03 (2.67)	0.03 (21.27)	0.03 (11.69)	0.03 (11.88)	0.04 (14.23)	0.02 (17.92)	0.00 (0.00)	0.0097
118	Dihydrobenzofuran 2	29.25 (4.84)	15.21 (14.42)	38.34 (6.53)	24.50 (25.19)	38.23 (19.96)	19.74 (30.52)	0.15 (23.37)	0.0003
119	1(3h)-Isobenzofuranone	0.89 (9.53)	0.88 (28.74)	0.77 (13.26)	0.83 (10.29)	1.15 (24.05)	0.98 (20.29)	0.00 (0.00)	0.2328
120	Ethylsyringol	0.24 (6.88)	0.12 (21.49)	0.35 (13.88)	0.61 (15.05)	0.44 (18.41)	0.19 (23.69)	0.00 (0.00)	0.0000
121	Pyridinol	4.25 (12.16)	3.42 (30.75)	3.33 (11.13)	3.08 (26.80)	3.21 (25.42)	5.60 (32.42)	0.00 (0.00)	0.0709
122	Benzoic acid	6.57 (16.95)	5.83 (13.65)	8.31 (14.79)	5.22 (2.65)	6.05 (26.25)	7.85 (22.47)	1.85 (11.15)	0.0606
123	3-Methoxyphenol	0.10 (4.25)	0.05 (25.14)	0.11 (9.16)	0.10 (14.89)	0.13 (15.33)	0.05 (22.72)	0.00 (0.00)	0.0000
124	2-Methyl-2,3-dihydrobenzofuran	1.14 (10.82)	0.64 (13.54)	1.14 (10.88)	2.14 (20.91)	1.14 (19.61)	2.61 (26.80)	0.00 (0.00)	0.0002
125	2,3-Dihydro-1H-inden-5-ol	3.01 (3.54)	1.83 (22.63)	2.25 (17.02)	3.08 (10.71)	3.76 (16.43)	2.13 (19.37)	0.00 (0.00)	0.0007
126	Indole	13.54 (9.03)	6.14 (20.74)	17.90 (10.38)	10.44 (14.94)	21.71 (3.70)	3.17 (33.56)	0.00 (0.00)	0.0000
127	Propylsyringol	0.04 (32.83)	0.01 (14.97)	0.07 (11.23)	0.12 (24.48)	0.09 (8.79)	0.02 (46.25)	0.00 (0.00)	0.0000
128	5-(Hydroxymethyl)-2-furaldehyde	0.02 (4.74)	0.03 (17.96)	0.02 (17.01)	0.02 (23.70)	0.01 (34.73)	0.07 (19.07)	0.00 (0.00)	0.0000
129	3-Methyl-1H-indole	10.46 (10.89)	5.67 (20.28)	12.06 (10.78)	8.67 (12.51)	15.77 (11.71)	7.03 (31.89)	0.00 (0.00)	0.0000
130	4-Propenylsyringol	0.04 (7.49)	0.02 (22.39)	0.05 (9.40)	0.08 (16.68)	0.06 (16.99)	0.02 (21.87)	0.00 (0.00)	0.0000
131	Benzeneacetic acid	6.57 (11.46)	5.06 (20.18)	6.33 (15.20)	7.13 (1.51)	5.26 (25.75)	6.47 (21.48)	3.28 (11.36)	0.1765
132	Vanillin	0.57 (7.31)	0.44 (8.74)	0.56 (14.23)	0.71 (23.42)	0.63 (26.34)	0.51 (23.78)	0.08 (7.74)	0.1406
133	Vanillic acid methyl ester	0.16 (5.32)	0.07 (10.28)	0.14 (10.27)	0.21 (14.95)	0.18 (16.72)	0.06 (22.98)	0.01 (5.51)	0.0000
134	Acetovanillone	1.47 (3.47)	0.89 (16.96)	1.42 (5.91)	1.34 (12.37)	1.63 (14.00)	0.83 (18.74)	0.00 (10.51)	0.0001
135	Guaiacyl acetone	0.59 (5.98)	0.40 (16.53)	0.68 (8.09)	0.77 (13.83)	0.69 (14.74)	0.41 (18.74)	0.00 (0.00)	0.0003

Peak no. [a]	Compound	Gar [b]	Ork [b]	Gle [b]	St F [b]	Cas [b]	Tom [b]	Unpeat [b]	p value [c]
136	Pyrocatechol	0.04 (9.01)	0.02 (10.64)	0.03 (43.21)	0.06 (53.99)	0.04 (43.70)	0.04 (56.52)	0.00 (0.00)	0.1982
137	Guaiacyl propanaldehyde	0.20 (5.44)	0.12 (16.77)	0.20 (3.54)	0.21 (10.53)	0.24 (12.39)	0.12 (16.13)	0.00 (13.38)	0.0000
138	Unknown phenolic	0.05 (10.22)	0.02 (8.51)	0.05 (21.51)	0.04 (20.81)	0.06 (29.79)	0.02 (28.35)	0.00 (0.00)	0.0007
139	Guaiacol	2.46 (7.28)	1.42 (21.21)	2.87 (5.40)	2.57 (13.29)	2.90 (9.62)	1.43 (13.98)	0.00 (0.00)	0.0000
140	Methylguaiacol	1.56 (7.82)	1.00 (20.26)	1.98 (5.57)	2.13 (15.23)	1.97 (13.12)	1.23 (15.34)	0.00 (0.00)	0.0001
141	*o*-Cresol	1.20 (2.55)	0.82 (26.67)	1.17 (6.55)	1.21 (11.84)	1.21 (9.42)	1.14 (12.14)	0.00 (0.00)	0.0269
142	Phenol	6.05 (6.08)	3.75 (28.42)	6.97 (4.09)	5.86 (9.80)	6.18 (9.62)	6.09 (12.80)	0.09 (7.43)	0.0016
143	Ethylguaiacol	1.75 (11.85)	1.14 (15.73)	2.12 (7.53)	1.93 (14.73)	2.23 (14.72)	1.32 (13.84)	0.00 (0.00)	0.0004
144	*p*-Cresol	1.76 (2.94)	1.02 (25.21)	1.99 (9.31)	1.82 (11.37)	1.98 (14.04)	1.66 (14.98)	0.00 (0.00)	0.0015
145	*m*-Cresol	0.74 (5.04)	0.50 (28.79)	0.62 (9.87)	0.94 (10.00)	0.78 (12.11)	0.78 (12.76)	0.00 (0.00)	0.0020
146	4-Ethylphenol	2.38 (3.12)	1.50 (19.65)	2.47 (10.19)	2.42 (10.73)	2.71 (14.77)	2.17 (14.27)	0.00 (0.00)	0.0037

[a] Colour coding indicates class of compound: carbohydrate-derived, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] Data are response ratios. Data in brackets are Relative Sandard Deviations.

[c] P values refer to1 way ANOVA of peated malt using peat location as a factor, non-significant compounds are highlighted red.

[d] Methylcyclopentanone is reported as response ratio x 10^{1}.

Appendix G

Table 3.12. Response ratios of peat-derived compounds detected in industrially produced peated malts. Data are the average of two injections. Letters after sample names indicate where more than one sample was obtained from the same maltings (for Orkney and Tomintoul a single sample was analysed in duplicate).

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
1	Pyridine	2.48	2.29	3.32	1.93	2.74	2.75	2.15	2.40	0.45	0.71	3.26	3.18	2.08	1.38	3.61	3.54
2	2-Methylcyclopentanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2-Methylpyridine	6.35	4.78	6.90	4.06	6.57	6.14	1.99	2.13	0.85	1.40	4.29	5.06	3.48	2.75	5.68	5.95
4	2,6-Lutidine	2.56	1.56	2.69	2.39	2.75	2.22	0.56	0.58	0.62	0.70	1.14	1.58	1.44	1.14	1.66	1.66
5	2-Methylpyrazine	0.44	0.53	0.78	0.45	0.59	0.54	0.51	0.47	0.08	0.14	0.94	0.77	0.32	0.30	0.81	0.83
6	2-Ethylpyridine	4.49	2.58	3.91	2.25	3.79	3.50	1.32	1.52	0.29	0.60	2.52	3.46	1.98	1.61	3.04	3.06
7	Ethylpicoline 1	3.77	1.96	3.49	2.62	3.54	2.87	0.52	0.51	0.31	0.59	1.27	1.89	1.50	1.35	1.83	1.83
8	Picoline 2	6.84	4.07	6.31	4.02	5.96	5.50	2.19	2.31	0.74	1.25	4.23	5.04	3.44	2.71	5.38	5.47
9	Picoline 3	2.96	1.62	2.85	2.26	2.73	2.36	0.67	0.63	0.42	0.60	1.50	1.78	1.63	1.32	2.02	2.04
10	Lutidine 2	6.05	3.16	5.77	4.88	6.67	5.47	1.18	1.09	0.85	1.32	2.54	4.01	3.14	2.80	3.47	3.28
11	Lutidine 3	4.09	2.01	3.99	3.56	4.91	4.00	1.18	1.02	1.26	1.09	1.95	3.25	2.51	2.06	2.34	2.27
12	Lutidine 4	3.15	1.50	2.88	2.46	3.48	2.82	0.79	0.74	0.80	0.74	1.43	2.32	1.56	1.39	1.67	1.59
13	2-Cyclopenten-1-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	Trimethylpyridine 1	0.65	0.31	0.68	0.44	0.79	0.68	0.24	0.22	0.23	0.17	0.32	0.50	0.29	0.30	0.31	0.35
15	2-Methyl-2-cyclopenten-1-one	0.07	0.09	0.13	0.13	0.07	0.08	0.04	0.04	0.04	0.04	0.09	0.08	0.11	0.08	0.22	0.18
16	Trimethylpyridine 2	0.74	0.47	0.66	0.33	0.76	0.59	0.46	0.42	0.02	0.11	0.79	0.74	0.46	0.57	0.70	0.66
17	3-Ethylpyridine	7.56	3.42	5.26	2.17	6.16	5.82	2.65	2.60	0.20	0.44	3.85	5.69	1.50	1.63	4.42	4.49
18	Ethylpicoline 2	6.82	2.97	5.61	2.92	6.99	5.94	1.24	1.23	0.14	0.31	2.60	4.30	1.26	1.57	3.04	3.10
19	4-Ethylpyridine	2.12	0.98	1.79	1.33	2.15	1.82	0.38	0.37	0.10	0.21	0.88	1.45	0.75	0.90	1.10	1.09
20	Ethylpicoline 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
21	Ethylpicoline 4	2.00	0.83	1.57	1.13	1.88	1.61	0.39	0.39	0.06	0.13	0.76	1.22	0.51	0.61	0.86	0.90
22	Ethylpicoline 5	4.77	1.94	4.56	2.76	5.48	4.54	0.96	0.82	0.11	0.22	1.87	3.18	1.13	1.65	2.01	2.22
23	Lutidine 5	4.13	1.75	3.87	2.81	4.60	4.08	1.00	0.95	0.21	0.37	1.85	3.16	1.62	2.16	2.24	2.42
24	Ethyllutidine	1.72	0.74	1.63	1.09	1.88	1.62	0.50	0.44	0.15	0.19	0.81	1.17	0.51	0.73	0.69	0.69
25	Acetic acid	0.04	0.07	0.07	0.08	0.04	0.07	0.05	0.06	0.04	0.07	0.12	0.08	0.06	0.04	0.09	0.08
26	Dimethylcyclopentenone 1	0.02	0.02	0.04	0.03	0.02	0.02	0.01	0.01	0.00	0.00	0.02	0.02	0.02	0.02	0.05	0.04
27	Furfural	0.04	0.09	0.11	0.10	0.07	0.08	0.03	0.04	0.03	0.03	0.15	0.09	0.34	0.11	0.30	0.28
28	Ethenylpyridine	1.75	1.14	1.64	0.80	1.81	1.75	1.50	1.46	0.10	0.19	2.30	2.10	0.72	0.62	1.52	1.57
29	Trimethylpyridine 3	1.13	0.52	1.38	0.94	1.41	1.21	0.25	0.19	0.17	0.18	0.50	0.88	0.60	0.59	0.45	0.49
30	Lutidine 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31	Dimethylcyclopentenone 2	0.04	0.04	0.06	0.06	0.04	0.04	0.01	0.01	0.00	0.00	0.05	0.05	0.04	0.04	0.12	0.10
32	Indene	0.35	0.53	0.83	0.62	0.59	0.65	0.17	0.16	0.05	0.07	0.68	0.52	0.42	0.22	0.82	0.76
33	Trimethylcyclopentenone 1	0.02	0.02	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.05	0.04
34	Benzofuran	0.11	0.14	0.22	0.16	0.15	0.16	0.10	0.10	0.04	0.06	0.23	0.23	0.18	0.14	0.26	0.26
35	2-Acetylfuran	0.10	0.15	0.23	0.20	0.13	0.13	0.07	0.07	0.05	0.06	0.15	0.13	0.32	0.16	0.55	0.48
36	Trimethylpyridine 4	3.21	1.80	3.50	1.41	3.82	3.83	1.44	1.32	0.16	0.09	2.21	2.42	0.61	0.97	1.67	2.27
37	3-Methyl-2-cyclopenten-1-one	0.19	0.20	0.29	0.29	0.20	0.22	0.09	0.09	0.07	0.09	0.25	0.25	0.22	0.18	0.60	0.50
38	2,3-Dimethylcyclopentene-1-one	0.33	0.31	0.47	0.44	0.36	0.36	0.15	0.14	0.11	0.14	0.36	0.39	0.35	0.30	0.98	0.84
39	3,4-Dimethylcyclopentene-1-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	Trimethylcyclopentenone 2	0.03	0.02	0.04	0.03	0.03	0.03	0.01	0.01	0.01	0.00	0.03	0.03	0.02	0.02	0.07	0.06
41	Dihydromethylenefuranone	0.02	0.03	0.04	0.04	0.03	0.03	0.02	0.02	0.02	0.02	0.04	0.03	0.05	0.03	0.10	0.08
42	5-Methylfurfural	0.17	0.24	0.37	0.32	0.27	0.26	0.15	0.15	0.10	0.10	0.25	0.23	1.42	0.39	1.23	1.31
43	3-Methoxypyridine	39.02	16.52	26.89	15.66	35.20	32.21	7.76	7.37	1.65	2.20	15.14	22.23	6.38	8.76	18.66	19.32
44	2-Methylbenzofuran	0.10	0.13	0.20	0.17	0.13	0.15	0.00	0.00	0.00	0.00	0.17	0.15	0.14	0.07	0.35	0.34
45	2-Acetyl-1-methylpyrrole	14.69	5.79	13.22	6.52	15.51	13.91	2.41	2.05	0.45	0.53	4.83	7.81	2.07	3.37	4.59	4.84
46	Benzonitrile	1.93	2.01	3.02	1.52	2.65	2.45	2.52	2.01	0.18	0.20	3.66	3.29	1.44	1.38	1.17	1.40

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
47	2-Acetyl-5-methylfuran	0.10	0.11	0.17	0.14	0.12	0.12	0.05	0.05	0.03	0.04	0.13	0.12	0.18	0.11	0.42	0.37
48	2,3-Dihydro-1H-indole	9.33	3.75	8.46	3.72	9.22	8.41	2.32	1.93	0.22	0.25	3.00	4.75	1.33	2.26	3.05	3.58
49	3-Ethyl-2-cyclopenten-1-one	0.18	0.15	0.27	0.22	0.20	0.23	0.09	0.08	0.05	0.06	0.23	0.25	0.15	0.18	0.40	0.39
50	Furfuryl alcohol	0.02	0.05	0.09	0.08	0.03	0.05	0.02	0.03	0.02	0.02	0.13	0.09	0.07	0.05	0.56	0.46
51	Benzeneacetaldehyde	2.10	1.84	2.84	1.82	2.35	3.17	1.13	0.98	0.29	0.50	4.41	3.59	1.53	2.33	3.02	3.09
52	Acetophenone	0.92	0.68	1.36	0.88	1.01	1.04	0.53	0.46	0.18	0.16	0.96	1.19	1.07	0.92	1.35	1.49
53	2-Methylbenzoxazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
54	5-Methyl-2-furanmethanol	0.04	0.04	0.14	0.08	0.03	0.05	0.03	0.03	0.04	0.04	0.11	0.08	0.08	0.04	0.38	0.32
55	3-Hydroxybenzonitrile	4.22	2.45	3.53	2.03	4.81	4.70	3.55	3.17	0.29	0.39	5.42	5.82	1.43	1.97	5.31	5.69
56	Methylfuranone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
57	Naphthalene	2.64	3.20	4.71	3.92	3.79	4.24	1.19	1.17	0.47	0.49	4.87	4.22	2.26	1.41	4.69	4.72
58	Pyridinecarbonitrile 1	1.60	1.17	1.83	0.98	2.05	2.13	1.08	1.19	0.03	0.01	2.49	2.26	0.56	0.68	1.71	1.85
59	Methylacetophenone 1	0.31	0.20	0.48	0.32	0.44	0.44	0.16	0.13	0.05	0.06	0.37	0.53	0.39	0.38	0.57	0.65
60	Methylacetophenone 2	0.71	0.45	0.71	0.53	0.79	0.79	0.45	0.38	0.22	0.21	0.68	0.96	0.49	0.62	0.70	0.83
61	Dimethoxytoluene 1	0.20	0.09	0.25	0.13	0.23	0.25	0.05	0.06	0.00	0.00	0.19	0.23	0.08	0.14	0.13	0.16
62	1-Phenylethanol	0.40	0.27	0.50	0.35	0.42	0.48	0.23	0.20	0.06	0.06	0.46	0.64	0.21	0.38	0.79	0.77
63	4-Acetylphenol	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02
64	Cyclotene	0.28	0.24	0.39	0.33	0.30	0.35	0.14	0.15	0.16	0.19	0.28	0.29	0.38	0.25	0.65	0.57
65	1-(3-Pyridinyl)-ethanone	1.39	0.74	1.55	0.62	1.87	1.73	1.80	1.46	0.00	0.00	1.81	1.85	0.59	0.95	0.87	1.27
66	2-Chlorophenol	0.03	0.14	0.15	0.14	0.07	0.09	0.00	0.00	0.00	0.00	0.17	0.13	0.04	0.00	0.25	0.21
67	2-Methylnaphthalene	1.42	1.29	1.82	1.61	1.83	2.05	0.48	0.45	0.18	0.16	2.09	2.10	0.91	0.63	2.25	2.37
68	Benzyl alcohol	1.18	1.02	1.23	1.08	1.18	1.32	1.47	1.43	0.90	0.92	2.22	3.21	1.00	1.08	1.52	1.62
69	2,6-Dimethylphenol	0.02	0.02	0.03	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.01	0.04	0.03
70	1-Methylnaphthalene	1.32	1.16	1.63	1.43	1.68	1.95	0.45	0.42	0.14	0.11	1.98	2.01	0.76	0.59	1.78	1.93
71	Pyridinecarbontrile 2	1.61	0.75	1.38	0.74	1.73	1.49	1.84	1.50	0.06	0.05	2.69	2.92	0.44	0.78	0.77	0.72
72	Hydroxymethylacetophenone	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01
73	Benzylnitrile	3.14	1.42	3.11	1.48	3.97	3.12	2.43	1.68	0.00	0.00	3.54	3.84	1.86	1.39	1.46	1.85

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
74	Methoxymethylphenol	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01
75	Quinoline	72.22	58.37	91.34	41.82	106.85	110.12	65.15	53.84	3.10	3.89	99.82	88.67	23.39	29.63	51.23	60.27
76	Dimethoxytoluene 2	0.19	0.05	0.27	0.15	0.26	0.27	0.00	0.00	0.00	0.00	0.15	0.16	0.14	0.16	0.17	0.22
77	Maltol	0.29	0.36	0.62	0.43	0.46	0.53	0.24	0.21	0.12	0.14	0.56	0.52	0.57	0.40	0.82	0.87
78	2-Acetylpyrrole	32.85	16.89	27.93	14.90	40.89	37.84	22.18	18.27	1.50	1.85	32.38	37.33	8.25	13.25	27.45	30.66
79	Methylquinoline 1	18.97	16.09	22.96	10.88	26.07	26.47	14.46	11.69	1.05	1.31	20.69	20.05	5.91	6.34	13.03	14.54
80	Biphenyl	0.63	0.47	0.70	0.64	0.80	0.96	0.37	0.35	0.09	0.08	1.23	1.38	0.46	0.42	0.92	1.08
81	Isoquinoline	21.42	19.33	27.17	12.77	30.43	32.36	24.31	19.44	1.44	1.72	29.20	27.26	7.82	9.17	14.99	17.22
82	Methylindanone 1	0.82	0.69	1.49	0.87	1.12	1.51	0.45	0.39	0.05	0.05	1.35	1.49	0.52	0.72	1.07	1.35
83	Methylindanone 2	1.07	0.79	1.90	1.05	1.44	1.90	0.57	0.50	0.12	0.00	1.42	1.53	0.81	1.05	1.88	2.31
84	2-Ethylphenol	0.08	0.06	0.09	0.07	0.07	0.08	0.02	0.02	0.01	0.01	0.07	0.07	0.04	0.04	0.10	0.09
85	3-Hydroxy-2,6-dimethyl-4H-pyran-4-one	0.06	0.07	0.11	0.08	0.08	0.09	0.03	0.02	0.02	0.01	0.06	0.06	0.06	0.04	0.08	0.08
86	Dimethylphenol 2 + 3	0.18	0.13	0.23	0.18	0.19	0.20	0.05	0.04	0.02	0.02	0.17	0.18	0.12	0.11	0.27	0.26
87	2,4-Dimethoxyphenol	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01
88	Dimethylquinoline	6.01	5.31	6.75	3.26	7.22	8.04	3.09	2.75	0.66	0.61	5.89	5.62	1.48	1.63	4.22	4.72
89	Propylguaiacol	0.03	0.02	0.04	0.03	0.03	0.03	0.01	0.01	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.03
90	5-Methyl-1H-pyrrole-2-carboxaldehyde	8.05	5.24	9.47	4.07	11.23	10.77	7.45	6.19	0.54	0.48	11.21	11.52	3.74	5.07	9.04	12.25
91	C3 phenol 1	0.05	0.03	0.06	0.04	0.04	0.05	0.01	0.01	0.01	0.01	0.04	0.04	0.02	0.02	0.04	0.04
92	C3 phenol 2	0.01	0.01	0.02	0.01	0.01	0.02	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02
93	Dimethylphenol 4	0.08	0.05	0.10	0.07	0.08	0.09	0.03	0.03	0.01	0.01	0.08	0.08	0.05	0.06	0.12	0.11
94	Methylquinoline 2	11.38	10.23	12.97	6.28	14.32	15.05	8.70	7.45	0.98	1.01	12.21	12.12	3.73	4.43	9.04	9.81
95	Methylindanone 3	1.66	1.57	2.79	1.66	2.27	2.74	1.09	0.91	0.30	0.25	2.14	2.33	1.37	1.77	2.71	3.16
96	C3 phenol 3	0.14	0.10	0.19	0.13	0.14	0.16	0.04	0.03	0.02	0.02	0.13	0.12	0.08	0.08	0.16	0.17
97	C3 phenol 4	0.02	0.02	0.03	0.02	0.02	0.03	0.01	0.01	0.00	0.00	0.02	0.02	0.06	0.02	0.03	0.03
98	Eugenol	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.02	0.01	0.01	0.01	0.01	0.01
99	3-Ethylphenol	0.20	0.13	0.26	0.17	0.19	0.21	0.08	0.07	0.03	0.03	0.20	0.20	0.13	0.16	0.21	0.22

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
100	Methylindanone 4	1.81	1.88	3.29	1.97	2.56	3.28	1.41	1.23	0.40	0.35	2.54	2.88	1.77	2.14	3.20	3.76
101	Vinylguaiacol	0.08	0.09	0.15	0.11	0.11	0.13	0.03	0.02	0.02	0.03	0.15	0.09	0.16	0.09	0.15	0.15
102	C3 phenol 5	0.04	0.03	0.06	0.03	0.04	0.05	0.01	0.01	0.01	0.00	0.04	0.03	0.03	0.03	0.05	0.06
103	C3 phenol 6	0.01	0.01	0.03	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.01	0.09	0.12
104	C3 phenol 7	0.07	0.05	0.09	0.06	0.06	0.07	0.02	0.02	0.01	0.01	0.06	0.06	0.04	0.04	0.07	0.07
105	4-Propylphenol	0.07	0.05	0.09	0.06	0.07	0.07	0.02	0.02	0.01	0.00	0.06	0.05	0.03	0.04	0.07	0.07
106	Propenylmethoxyphenol 1	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01
107	Syringol	0.29	0.34	0.55	0.38	0.39	0.41	0.07	0.06	0.07	0.09	0.23	0.24	0.22	0.23	0.15	0.15
108	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	0.08	0.10	0.12	0.10	0.12	0.13	0.04	0.03	0.00	0.03	0.12	0.14	0.11	0.07	0.19	0.19
109	Dibenzofuran	2.88	2.91	3.94	3.04	3.91	4.88	2.14	2.03	0.52	0.40	5.13	5.34	2.68	2.44	5.02	6.08
110	Propenylmethoxyphenol 2	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
111	Propenylphenol 1	0.01	0.01	0.02	0.01	0.01	0.02	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.01	0.01	0.01
112	1-Indolinecarboxaldehyde	9.64	7.65	10.44	4.70	10.85	11.17	6.22	5.10	0.71	0.64	8.21	8.45	2.43	3.49	4.45	4.92
113	Methylsyringol	0.10	0.12	0.22	0.15	0.15	0.16	0.01	0.01	0.03	0.04	0.09	0.08	0.12	0.11	0.11	0.10
114	Propenylmethoxyphenol 3	0.01	0.01	0.03	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.01	0.03	0.03
115	2,3-Dihydrobenzofuran	4.76	4.21	8.56	4.48	7.30	8.41	3.38	2.77	0.75	0.67	8.43	8.74	4.29	4.76	4.27	5.18
116	4-Butylphenol	0.03	0.03	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.01	0.03	0.03	0.02	0.03	0.02	0.02
117	Propenylphenol 2	0.02	0.02	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.00	0.02	0.02	0.02	0.01	0.02	0.02
118	Dihydrobenzofuran 2	16.42	16.12	32.35	16.61	26.26	33.40	3.68	2.81	3.62	3.60	32.65	27.43	17.15	11.55	11.88	13.64
119	1(3h)-Isobenzofuranone	1.27	1.28	1.93	1.05	1.57	2.12	1.73	1.50	0.29	0.23	2.49	2.71	0.89	1.26	1.10	1.26
120	Ethylsyringol	0.09	0.10	0.19	0.13	0.12	0.13	0.01	0.01	0.03	0.03	0.06	0.06	0.07	0.07	0.09	0.09
121	Pyridinol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
122	Benzoic acid	6.66	7.51	9.19	4.50	8.32	12.81	9.54	5.19	0.22	0.19	20.75	18.71	1.49	3.67	7.08	8.80
123	3-Methoxyphenol	0.04	0.04	0.06	0.04	0.04	0.05	0.02	0.01	0.01	0.01	0.04	0.04	0.02	0.03	0.02	0.02
124	2-Methyl-2,3-dihydrobenzofuran	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
125	2,3-Dihydro-1H-inden-5-ol	5.45	3.53	6.83	3.50	5.16	6.00	1.29	1.26	0.42	0.19	2.57	3.57	1.42	2.51	2.20	2.60

Peak no. [a]	Compound	Cas a	Cas b	Cas c	Cas d	Cas e	Cas if	Ork a	Ork b	Gar a	Gar b	Gle a	Gle b	StF a	StF b	Tom a	Tom b
126	Indole	28.57	19.36	33.24	14.65	42.73	39.17	4.82	3.52	2.34	2.76	23.27	19.93	16.23	12.10	14.57	18.52
127	Propylsyringol	0.03	0.03	0.05	0.04	0.03	0.04	0.00	0.00	0.01	0.01	0.02	0.02	0.01	0.01	0.03	0.03
128	5-(Hydroxymethyl)-2-furaldehyde	0.28	0.37	0.34	0.36	0.39	0.35	0.44	0.44	0.30	0.29	0.67	0.51	0.45	0.34	0.45	0.36
129	3-Methyl-1H-indole	24.63	13.99	28.77	12.18	32.34	29.22	7.72	5.77	1.03	0.54	17.59	18.42	6.26	8.16	15.32	17.78
130	4-Propenylsyringol	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01
131	Benzeneacetic acid	6.68	7.42	9.76	4.08	9.02	13.08	5.92	4.91	0.06	0.01	19.35	17.62	0.95	2.65	8.61	11.26
132	Vanillin	0.44	0.58	0.54	0.53	0.45	0.51	0.84	0.82	0.38	0.40	0.75	0.77	0.42	0.59	0.41	0.41
133	Vanillic acid methyl ester	0.08	0.10	0.11	0.09	0.08	0.09	0.06	0.06	0.03	0.03	0.12	0.10	0.03	0.06	0.04	0.04
134	Acetovanillone	0.58	0.75	0.86	0.67	0.58	0.71	0.43	0.40	0.26	0.25	0.74	0.68	0.35	0.52	0.36	0.35
135	Guaiacyl acetone	0.18	0.25	0.31	0.25	0.20	0.26	0.12	0.11	0.11	0.13	0.30	0.26	0.19	0.19	0.18	0.18
136	Pyrocatechol	0.02	0.04	0.13	0.03	0.06	0.08	0.01	0.01	0.00	0.01	0.03	0.02	0.04	0.02	0.04	0.04
137	Guaiacyl propanaldehyde	0.10	0.12	0.14	0.12	0.09	0.12	0.05	0.05	0.04	0.04	0.11	0.09	0.05	0.08	0.06	0.06
138	Unknown phenolic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
139	Guaiacol	0.42	0.34	0.59	0.41	0.46	0.48	0.12	0.11	0.08	0.07	0.38	0.37	0.33	0.31	0.32	0.35
140	Methylguaiacol	0.19	0.15	0.31	0.21	0.24	0.23	0.06	0.06	0.04	0.03	0.19	0.16	0.20	0.19	0.18	0.20
141	*o*-Cresol	0.55	0.51	0.84	0.58	0.65	0.69	0.19	0.19	0.09	0.09	0.68	0.66	0.53	0.44	0.99	1.03
142	Phenol	2.54	2.71	4.31	2.96	3.23	3.60	1.12	1.14	0.51	0.54	4.29	4.02	2.94	2.28	4.52	4.84
143	Ethylguaiacol	0.28	0.20	0.44	0.28	0.33	0.32	0.08	0.06	0.05	0.04	0.23	0.22	0.24	0.19	0.25	0.27
144	*p*-Cresol	0.89	0.83	1.40	0.93	1.07	1.18	0.30	0.31	0.12	0.13	1.09	1.05	0.71	0.64	1.08	1.13
145	*m*-Cresol	0.39	0.35	0.64	0.43	0.47	0.52	0.15	0.15	0.07	0.06	0.49	0.48	0.43	0.38	0.62	0.65
146	4-Ethylphenol	1.25	1.03	1.87	1.26	1.44	1.51	0.40	0.39	0.21	0.20	1.39	1.37	0.90	0.83	1.38	1.44

[a] Colour coding indicates class of compound: carbohydrate-derived, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics.

[b] Carbohydrate derivatives are reported as response ratio x 10^1.

[c] 2-Chlorophenol is reported as response ratio x 10^2.

Appendix H

Table 3.16. Compounds detected in lab-scale new-make spirit using GC-MS.

Peak no. [a]	Compound	RT	Estimated RI [b]	Literature RI [c]	MW	QI
1	Pyridine	11.28	1164	**1192**	79	79
2	2-Methylcyclopentanone	12.01	1190		98	69, 98
3	2-Methylpyridine	12.24	1198	1221	93	66, 93
4	2-Methylpyrazine	13.69	1249	1267	94	67, 94
5	2-Ethylpyridine	14.11	1264	1282	107	106, 107
6	Ethylcyclopentanone	14.18	1266		112	56, 84
7	Ethylpicoline 1	14.50	1277		121	120, 121
8	Unknown (55,98)	14.58	1280		?	55, 98
9	Picoline 2	14.74	1286		93	66, 93
10	Picoline 3	15.04	1296		93	66, 93
11	Unknown (95,140)	16.16	1336		?	95, 140
12	Ethylmethylpyrazine	17.36	1378		122	121, 122
13	3-Ethylpyridine	17.46	1381	1387	107	92, 107
14	Diethoxymethylfuran	19.42	1450		170	97, 125
15	Dimethylcyclopentenone	19.53	1454		110	67, 110
16	Furfural	19.81	1464	**1468**	96	96
17	Ethenylpyridine	20.47	1487		105	104, 105
18	Trimethylcyclopentenone 1	21.13	1510		124	109, 124
19	2-acetylfuran	21.19	1512	1487	110	95, 110
20	3-Methyl-2-cyclopenten-1-one	22.09	1544	1535	96	67, 96
21	2,3-Dimethylcyclopentene-1-one	22.64	1563		110	67, 110
22	Trimethylcyclopentenone 2	23.03	1577		124	109, 124
23	5-Methylfurfural	23.40	1590	**1568**	110	109, 110
24	3-methoxypyridine	23.77	1603		109	66, 109
25	Benzonitrile	24.50	1628		103	103
26	2-Acetyl-5-methylfuran	24.68	1635	1848	124	109, 124
27	Furfuryl alcohol	25.56	1666	**1665**	98	98
28	Benzeneacetaldehyde	25.60	1667	1642	120	91, 120
29	Acetophenone	25.90	1678	**1653**	120	77, 105
30	Methylfuranylpropanone	26.63	1703		138	109, 138
31	5-Methyl-2-furanmethanol	27.37	1729		112	95, 112
32	Naphthalene	28.60	1772	**1735**	128	128
33	Methylacetophenone 1	28.91	1783		134	91, 119, 134
34	Methlacetophenone 2	29.57	1807		134	119, 134
35	Dimethoxytoluene 1	29.95	1820		152	137, 152
36	4-acetylphenol	30.30	1832		136	121, 136
37	2-Chlorophenol	31.16	1862		128	64, 128

Peak no. [a]	Compound	RT	Estimated RI [b]	Literature RI [c]	MW	QI
38	Unknown (123,152)	31.27	1866		?	123, 152
39	Guaiacol	31.56	1876	**1868**	124	109, 124
40	2-methylnaphthalene	31.79	1884	1876	142	141, 142
41	Unknown (123,138)	31.85	1887		?	123, 138
42	2,6-dimethylphenol	32.78	1919	1889	122	107, 122
43	1-methylnaphthalene	32.78	1919	1915	142	141, 142
44	Hydroxymethylacetophenone	33.41	1941		150	135, 150
45	Benzylnitrile	33.60	1948	1894	117	90, 117
46	Unknown (135, 150)	33.66	1950		?	135, 150
47	Methoxymethylphenol	33.78	1954		138	123, 138
48	Methylguaiacol	34.13	1967	**1968**	138	123, 138
49	Quinoline	34.24	1970		129	129
50	Dimethoxytoluene 2	34.38	1975		152	137, 152
51	*o*-Cresol	35.10	2001	**2026**	108	108
52	Phenol	35.22	2005	**1994**	94	94
53	Biphenyl	35.28	2007		154	154
54	Ethylguaiacol	36.00	2032	**2034**	152	137, 152
55	Indanone	36.24	2041		132	104, 132
56	Methylindanone 2	36.51	2050		146	117, 146
57	2-Ethylphenol	36.75	2059		122	107, 122
58	Dimethylphenol 2 + 3	37.00	2067		122	122
59	*p*-Cresol	37.16	2073	**2079**	108	108
60	*m*-Cresol	37.36	2080	**2095**	108	108
61	Propylguaiacol	38.00	2103	**2138**	166	137, 166
62	C3 phenol 1	38.43	2118		136	121, 136
63	C3 phenol 2	38.61	2124		136	121, 136
64	Dimethylphenol 4	38.78	2130		122	107, 122
65	C3 phenol 3	39.13	2142		136	121, 136
66	C3 phenol 4	39.26	2147		136	121, 136
67	4-Ethylphenol	39.43	2153	**2095**	122	107, 122
68	Eugenol	39.43	2153	**2169**	164	149, 164
69	3-Ethylphenol	39.60	2159	2221	122	107, 122
70	Vinylguaiacol	40.10	2176	**2186**	150	135, 150
71	C3 phenol 5	40.26	2182		136	121, 136
72	Dibenzofuran	42.30	2254		168	139, 168

[a] Colour coding indicates class of compound: carbohydrate derivatives, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics and unknowns.

[b] Estimated RI were calculated as described in Chapter 2.4.2 using literature RI data for selected compounds (identified in bold).

[c] Literature RI data taken from [153,154,158-162].

Appendix I

Table 3.17. Response ratios (analyte peak areas relative to internal standard peak areas) of peat-derived compounds detected in lab-scale peated new-make spirits.

Peak no. [a]	Compound	Cas [b]	Gar [b]	Gle [b]	Ork [b]	StF [b]	Tom [b]	UP [b]	P value [c]
1	Pyridine	13.28 (3.07)	13.99 (17.17)	14.08 (5.14)	11.80 (10.56)	11.39 (6.87)	9.85 (2.17)	0.30 (16.98)	0.0059
2	2-Methylcyclopentanone	26.90 (4.99)	25.82 (11.21)	26.6 (8.35)	26.52 (3.93)	25.02 (8.74)	32.51 (8.95)	0.04 (173.21)	0.0169
3	2-Methylpyridine	6.98 (3.79)	6.81 (13.68)	7.42 (5.36)	5.13 (14.45)	4.90 (10.46)	4.01 (7.06)	0.00 (0.00)	0.0000
4	2-Methylpyrazine	4.47 (3.29)	3.95 (8.56)	3.95 (1.55)	2.85 (3.66)	3.47 (8.43)	2.30 (3.75)	0.00 (0.00)	0.0000
5	2-Ethylpyridine	1.49 (2.92)	1.48 (13.41)	1.56 (5.77)	1.10 (12.05)	0.97 (10.48)	0.80 (6.37)	0.00 (0.00)	0.0000
6	Ethylcyclopentanone	1.79 (4.69)	1.87 (15.96)	1.79 (8.97)	2.04 (3.56)	1.63 (11.19)	1.97 (12.97)	0.06 (90.24)	0.1987
7	Ethylpicoline 1	0.58 (1.51)	0.60 (15.49)	0.66 (7.08)	0.44 (15.81)	0.35 (10.76)	0.25 (5.92)	0.00 (0.00)	0.0000
8	Unknown (55,98)	3.16 (4.69)	3.31 (10.00)	2.97 (3.58)	3.58 (4.74)	2.32 (10.54)	2.90 (3.16)	0.00 (0.00)	0.0001
9	Picoline 2	4.45 (3.57)	4.51 (14.06)	4.68 (5.1)	3.58 (12.45)	3.62 (8.47)	3.05 (7.20	0.00 (0.00)	0.0008
10	Picoline 3	1.11 (5.58)	1.08 (14.05)	1.12 (6.8)	0.81 (16.24)	0.82 (9.46)	0.61 (10.53)	0.00 (0.00)	0.0002
11	Unknown (95,140)	32.08 (2.77)	34.32 (9.22)	36.79 (4.93)	56.92 (2.95)	32.8 (11.77)	74.64 (4.85)	0.23 (0.00)	0.0000
12	Ethylmethylpyrazine	0.96 (3.14)	0.79 (8.01)	0.79 (3.31)	0.52 (4.74)	0.58 (5.02)	0.49 (8.73)	0.04 (58.9)	0.0000
13	3-Ethylpyridine	0.78 (4.45)	0.75 (12.08)	0.77 (7.32)	0.55 (12.59)	0.56 (5.74)	0.42 (11.26)	0.00 (0.00)	0.0000
14	Diethoxymethylfuran	5.86 (11.65)	5.29 (21.82)	5.72 (11.35)	5.50 (18.61)	4.56 (19.1)	4.53 (37.01)	3.40 (4.77)	0.5294
15	Dimethylcyclopentenone	0.38 (4.92)	0.39 (14.53)	0.36 (5.54)	0.40 (10.27)	0.33 (17.81)	0.33 (11.34)	0.02 (58.38)	0.2311
16	Furfural	48.07 (5.75)	47.98 (14.56)	50.6 (7.81)	47.43 (0.63)	43.71 (10.09)	45.16 (5.90)	32.00 (4.93)	0.4246
17	Ethenylpyridine	0.40 (4.33)	0.38 (13.67)	0.41 (3.41)	0.27 (11.05)	0.25 (7.64)	0.20 (6.47)	0.00 (0.00)	0.0000
18	Trimethylcyclopentenone 1	1.10 (3.40)	1.07 (10.97)	1.14 (3.98)	1.11 (6.89)	0.89 (7.64)	1.06 (6.58)	0.00 (0.00)	0.0190
19	2-acetylfuran	15.03 (3.62)	15.81 (10.20)	17.70 (3.32)	19.04 (4.63)	13.6 (7.94)	30.36 (3.62)	0.01 (56.01)	0.0000
20	3-Methyl-2-cyclopenten-1-one	1.20 (3.89)	1.05 (4.01)	1.07 (4.91)	1.09 (1.55)	1.09 (3.59)	1.28 (7.87)	0.14 (8.43)	0.0016
21	2,3-Dimethylcyclopentene-1-one	6.58 (3.70)	6.32 (4.68)	6.70 (4.77)	6.64 (4.44)	5.95 (1.90)	7.52 (3.87)	0.00 (0.00)	0.0003
22	Trimethylcyclopentenone 2	0.73 (1.98)	0.74 (7.54)	0.80 (3.58)	0.85 (5.69)	0.68 (4.56)	0.81 (3.73)	0.00 (0.00)	0.0013
23	5-Methylfurfural	1.21 (2.55)	1.31 (11.54)	1.42 (4.53)	1.42 (1.72)	1.04 (7.00)	2.29 (2.84)	0.28 (2.30)	0.0000

Peak no. [a]	Compound	Cas [b]	Gar [b]	Gle [b]	Ork [b]	StF [b]	Tom [b]	UP [b]	P value [c]
24	3-methoxypyridine	1.94 (4.60)	1.71 (5.16)	1.82 (5.51)	0.81 (4.48)	1.27 (5.47)	0.75 (8.36)	0.00 (0.00)	0.0000
25	Benzonitrile	4.04 (2.42)	4.52 (14.33)	4.35 (5.74)	3.93 (7.33)	3.42 (9.41)	2.47 (5.22)	0.73 (86.80)	0.0001
26	2-Acetyl-5-methylfuran	3.33 (4.25)	3.71 (7.99)	4.17 (3.57)	4.28 (5.36)	3.01 (7.23)	5.91 (3.07)	0.00 (0.00)	0.0000
27	Furfuryl alcohol	4.23 (0.73)	4.77 (5.66)	5.33 (2.58)	6.75 (0.59)	4.57 (5.09)	8.13 (7.43)	1.13 (7.19)	0.0000
28	Benzeneacetaldehyde	6.81 (9.77)	5.63 (17.15)	6.14 (6.95)	6.23 (12.02)	5.03 (11.41)	5.62 (15.06)	3.01 (8.12)	0.1296
29	Acetophenone	8.43 (2.88)	9.44 (9.51)	9.40 (3.80)	9.48 (4.48)	6.61 (6.56)	9.74 (3.96)	1.58 (41.12)	0.0000
30	Methylfuranylpropanone	0.46 (2.57)	0.48 (9.39)	0.57 (1.56)	0.58 (7.15)	0.39 (2.55)	0.80 (2.20)	0.00 (0.00)	0.0000
31	5-Methyl-2-furanmethanol	1.49 (5.63)	1.50 (6.30)	1.69 (9.37)	2.32 (3.73)	1.33 (8.57)	3.49 (6.34)	0.00 (0.00)	0.0000
32	Naphthalene	0.37 (1.84)	0.43 (3.65)	0.44 (9.84)	0.51 (5.34)	0.33 (5.77)	0.40 (0.66)	0.00 (0.00)	0.0000
33	Methylacetophenone 1	1.62 (5.94)	1.92 (9.53)	2.01 (6.02)	2.06 (6.14)	1.39 (4.67)	2.30 (5.31)	0.00 (0.00)	0.0000
34	Methlacetophenone 2	1.20 (1.77)	1.35 (9.51)	1.42 (3.01)	1.31 (3.92)	1.12 (5.40)	1.44 (3.47)	0.00 (0.00)	0.0005
35	Dimethoxytoluene 1	1.06 (2.05)	0.73 (5.51)	0.93 (3.71)	0.41 (1.75)	1.46 (1.74)	0.36 (3.14)	0.00 (0.00)	0.0000
36	4-acetylphenol	2.79 (3.04)	3.15 (9.26)	3.39 (3.21)	3.06 (4.60)	2.32 (4.43)	3.48 (3.81)	0.00 (0.00)	0.0000
37	2-Chlorophenol	0.83 (4.26)	0.77 (8.70)	1.14 (2.77)	0.69 (4.56)	0.37 (6.32)	1.40 (3.22)	0.00 (0.00)	0.0000
38	Unknown (123,152)	0.28 (3.23)	0.21 (14.15)	0.28 (6.56)	0.14 (2.91)	0.39 (0.73)	0.15 (4.51)	0.00 (0.00)	0.0000
39	Guaiacol	93.08 (3.10)	71.76 (4.61)	91.79 (4.52)	44.24 (2.70)	70.95 (4.68)	43.33 (4.19)	0.18 (9.47)	0.0000
40	2-methylnaphthalene	0.49 (3.55)	0.53 (3.98)	0.59 (13.58)	0.66 (4.99)	0.37 (9.49)	0.42 (3.20)	0.00 (0.00)	0.0000
41	Unknown (123,138)	2.23 (6.55)	1.36 (12.60)	2.18 (10.18)	0.72 (4.48)	2.13 (11.65)	0.95 (18.98)	0.00 (0.00)	0.0000
42	2,6-dimethylphenol	4.22 (4.41)	3.83 (7.95)	4.47 (6.31)	2.92 (4.62)	3.47 (6.88)	3.21 (4.40)	0.00 (0.00)	0.0000
43	1-methylnaphthalene	0.45 (7.23)	0.49 (3.59)	0.52 (12.60)	0.62 (1.95)	0.33 (8.04)	0.42 (1.53)	0.00 (0.00)	0.0000
44	Hydroxymethylacetophenone	0.79 (3.73)	0.82 (8.43)	0.91 (3.23)	0.74 (5.30)	0.58 (1.93)	0.90 (3.32)	0.00 (0.00)	0.0000
45	Benzylnitrile	3.18 (3.32)	2.54 (6.38)	3.05 (3.66)	1.66 (3.47)	1.80 (4.30)	1.59 (5.24)	0.00 (0.00)	0.0000
46	Unknown (135, 150)	0.41 (4.67)	0.44 (5.62)	0.46 (4.63)	0.47 (4.67)	0.37 (1.38)	0.54 (3.38)	0.00 (0.00)	0.0000
47	Methoxymethylphenol	2.3 (4.15)	1.51 (6.13)	2.05 (8.42)	0.85 (2.70)	2.28 (5.30)	0.91 (8.94)	0.00 (0.00)	0.0000
48	Methylguaiacol	46.27 (4.72)	32.00 (6.44)	45.55 (7.93)	20.65 (3.94)	42.77 (5.57)	25.87 (10.69)	0.00 (0.00)	0.0000
49	Quinoline	0.24 (7.31)	0.27 (6.96)	0.24 (10.97)	0.22 (7.27)	0.22 (9.80)	0.21 (11.11)	0.00 (0.00)	0.0256
50	Dimethoxytoluene 2	1.13 (10.42)	0.69 (7.76)	1.06 (10.36)	0.43 (6.40)	1.28 (10.24)	0.61 (22.94)	0.00 (0.00)	0.0000
51	*o*-Cresol	23.59 (3.53)	21.9 (4.78)	24.40 (4.49)	16.11 (3.03)	20.58 (3.44)	20.61 (2.67)	0.00 (0.00)	0.0000

Peak no. [a]	Compound	Cas [b]	Gar [b]	Gle [b]	Ork [b]	StF [b]	Tom [b]	UP [b]	P value [c]
52	Phenol	55.33 (3.42)	52.02 (3.12)	65.06 (5.33)	36.4 (1.37)	46.60 (2.40)	52.58 (7.76)	0.19 (24.36)	0.0000
53	Biphenyl	0.27 (5.16)	0.30 (3.02)	0.32 (13.00)	0.41 (1.55)	0.26 (9.13)	0.34 (3.87)	0.00 (0.00)	0.0000
54	Ethylguaiacol	44.3 (5.18)	30.28 (7.24)	41.79 (7.42)	20.34 (3.21)	33.36 (4.90)	23.24 (9.06)	0.00 (0.00)	0.0000
55	Indanone	1.00 (6.01)	1.08 (1.47)	1.02 (7.06)	1.17 (3.14)	1.00 (4.31)	1.38 (11.04)	0.00 (0.00)	0.0004
56	Methylindanone 2	0.57 (4.10)	0.60 (6.66)	0.64 (7.16)	0.70 (3.95)	0.60 (2.05)	0.82 (3.79)	0.00 (0.00)	0.0000
57	2-Ethylphenol	7.76 (3.97)	6.98 (4.32)	7.55 (4.90)	5.10 (2.33)	5.48 (3.44)	4.68 (1.72)	0.00 (0.00)	0.0000
58	Dimethylphenol 2 + 3	11.80 (4.45)	10.08 (4.70)	11.48 (5.44)	7.18 (2.47)	10.25 (2.61)	9.27 (2.47)	0.00 (0.00)	0.0000
59	*p*-Cresol	22.88 (3.80)	19.11 (4.06)	24.19 (5.53)	12.20 (1.48)	17.88 (1.83)	18.07 (7.40)	0.00 (0.00)	0.0000
60	*m*-Cresol	8.91 (3.67)	7.80 (2.91)	7.46 (6.07)	5.85 (1.63)	9.24 (1.66)	8.45 (7.94)	0.00 (0.00)	0.0000
61	Propylguaiacol	2.69 (6.17)	1.59 (7.87)	2.36 (7.71)	0.99 (6.12)	2.92 (4.23)	1.15 (8.46)	0.03 (88.96)	0.0000
62	C3 phenol 1	2.38 (4.63)	1.91 (7.67)	2.16 (7.46)	1.30 (1.61)	1.59 (3.24)	1.17 (2.86)	0.00 (0.00)	0.0000
63	C3 phenol 2	0.69 (6.38)	0.64 (13.26)	0.60 (7.88)	0.45 (6.96)	0.57 (8.87)	0.42 (7.08)	0.00 (0.00)	0.0002
64	Dimethylphenol 4	2.62 (4.00)	2.38 (3.65)	2.29 (5.82)	1.97 (2.59)	2.72 (3.42)	2.33 (4.38)	0.00 (0.00)	0.0000
65	C3 phenol 3	5.77 (5.27)	4.71 (5.46)	5.24 (6.81)	3.08 (1.59)	4.55 (7.82)	3.57 (0.21)	0.00 (0.00)	0.0000
66	C3 phenol 4	0.91 (8.49)	0.96 (22.96)	0.92 (10.49)	0.67 (12.01)	1.09 (30.77)	0.73 (25.79)	0.00 (0.00)	0.1491
67	4-Ethylphenol	37.20 (4.76)	30.10 (3.63)	35.38 (6.44)	20.70 (1.16)	28.67 (4.38)	26.99 (6.07)	0.57 (4.87)	0.0000
68	Eugenol	1.01 (4.31)	0.58 (5.50)	0.78 (9.03)	0.35 (5.51)	1.18 (5.10)	0.40 (9.23)	0.00 (0.00)	0.0000
69	3-Ethylphenol	4.20 (5.14)	3.51 (3.93)	3.02 (7.03)	2.21 (2.06)	3.83 (3.80)	2.69 (6.44)	0.64 (35.52)	0.0000
70	Vinylguaiacol	0.87 (6.87)	0.66 (3.69)	0.81 (21.02)	0.48 (4.59)	0.70 (6.52)	0.46 (9.28)	0.08 (87.23)	0.0001
71	C3 phenol 5	0.78 (0.04)	0.68 (0.04)	0.69 (0.05)	0.60 (0.01)	0.81 (0.03)	0.77 (0.03)	0.00 (0.00)	0.0001
72	Dibenzofuran	0.29 (6.78)	0.35 (0.67)	0.30 (10.01)	0.41 (4.37)	0.55 (9.28)	0.44 (1.12)	0.00 (0.00)	0.0000

[a] Colour coding indicates class of compound: carbohydrate derivatives, guaiacols, syringols, non specific phenols, nitrogen-containing compounds, aromatics, unknown.

[b] Data are response ratios x 10^2 and are the means of three new-make spirit samples (data for each sample was recorded as the mean of two injections) . Data in brackets are % RSDs.

[c] P values refer to ANOVA of peated new-make spirits using peat source as a factor, non-significant compounds are highlighted red.

Appendix J

Table 3.19. Response ratios of peat-derived compounds detected in industrial peated new-make spirits.

Peak no. [a]	Compound	Islay a [b]	Islay b [b]	Islay c [b]	Islay d [b]	Islay e [b]	Islay f [b]	Orkney a [b]	Orkney b [b]	St F a [b]	St F b [b]	St F f [b]	St F g [b]
1	Pyridine	7.39	10.97	7.15	3.31	8.10	3.08	1.17	0.97	2.88	0.91	5.55	3.08
2	2-Methylcyclopentanone	3.68	3.10	4.11	2.46	2.16	1.03	0.20	0.17	2.49	1.41	2.63	3.79
3	2-Methylpyridine	7.89	12.05	5.18	1.42	9.83	1.92	0.42	0.29	1.13	0.10	3.73	2.01
4	2-Methylpyrazine	2.10	2.12	2.26	1.65	1.71	0.82	0.20	0.17	1.49	0.96	1.51	1.30
5	2-Ethylpyridine	2.17	3.10	1.70	0.69	2.33	0.37	0.12	0.09	0.46	0.05	0.91	0.46
6	Ethylcyclopentanone	0.12	0.06	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.13
7	Ethylpicoline 1	0.68	0.74	0.35	0.03	0.79	0.17	0.01	0.00	0.06	0.00	0.29	0.18
8	Unknown (55,98)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	Picoline 2	3.85	5.69	2.77	0.90	4.79	1.12	0.33	0.26	0.80	0.12	2.00	1.29
10	Picoline 3	0.52	0.77	0.35	0.07	0.80	0.21	0.04	0.03	0.08	0.01	0.40	0.24
11	Unknown (95,140)	2.48	1.14	3.81	1.67	1.76	0.92	0.82	0.61	3.07	2.64	3.50	2.97
12	Ethylmethylpyrazine	0.55	0.51	0.57	0.38	0.40	0.09	0.00	0.00	0.29	0.14	0.25	0.24
13	3-Ethylpyridine	0.93	1.28	0.66	0.24	1.11	0.21	0.06	0.04	0.20	0.00	0.40	0.27
14	Diethoxymethylfuran	1.82	2.94	2.21	3.15	0.73	1.94	1.32	1.04	1.74	2.94	3.00	1.68
15	Dimethylcyclopentenone	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
16	Furfural	48.07	51.43	35.97	56.13	20.04	44.14	24.23	22.80	39.73	55.79	46.57	43.85

Peak no. [a]	Compound	Islay a [b]	Islay b [b]	Islay c [b]	Islay d [b]	Islay e [b]	Islay f [b]	Orkney a [b]	Orkney b [b]	St F a [b]	St F b [b]	St F f [b]	St F g [b]
17	Ethenylpyridine	0.47	0.56	0.47	0.24	0.47	0.10	0.00	0.00	0.18	0.08	0.19	0.21
18	Trimethylcyclopentenone 1	0.21	0.24	0.24	0.17	0.13	0.02	0.00	0.00	0.26	0.09	0.18	0.19
19	2-acetylfuran	4.83	4.67	3.96	3.71	2.55	2.31	0.82	0.67	4.08	2.38	4.46	4.39
20	3-Methyl-2-cyclopenten-1-one	0.24	0.22	0.18	0.20	0.27	0.00	0.00	0.00	0.32	0.11	0.19	0.12
21	2,3-Dimethylcyclopentene-1-one	2.11	2.08	1.85	1.36	1.67	0.48	0.13	0.08	2.19	0.89	1.71	1.53
22	Trimethylcyclopentenone 2	0.15	0.17	0.18	0.10	0.09	0.00	0.00	0.00	0.20	0.00	0.09	0.12
23	5-Methylfurfural	0.98	0.95	0.62	1.05	0.29	0.78	0.37	0.26	0.73	0.61	0.83	0.83
24	3-methoxypyridine	1.08	1.16	0.78	0.31	1.24	0.21	0.00	0.00	0.26	0.00	0.48	0.39
25	Benzonitrile	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	2-Acetyl-5-methylfuran	1.32	1.55	1.57	1.92	1.50	0.80	0.84	0.66	2.49	2.13	1.78	1.53
27	Furfuryl alcohol	2.23	1.22	1.68	1.84	1.10	2.45	1.08	0.81	2.30	1.66	2.56	2.65
28	Benzeneacetaldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	Acetophenone	1.67	1.58	1.98	1.07	1.21	0.55	0.23	0.16	1.91	0.74	1.87	2.09
30	Methylfuranylpropanone	0.15	0.16	0.15	0.09	0.08	0.00	0.00	0.00	0.20	0.03	0.17	0.19
31	5-Methyl-2-furanmethanol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.06
32	Naphthalene	3.13	3.15	3.26	1.77	1.88	0.60	0.21	0.21	1.61	0.86	1.59	1.89
33	Methylacetophenone 1	0.57	0.55	0.59	0.28	0.35	0.02	0.00	0.00	0.81	0.22	0.65	0.67
34	Methlacetophenone 2	0.43	0.29	0.43	0.19	0.25	0.00	0.00	0.00	0.45	0.16	0.35	0.51
35	Dimethoxytoluene 1	0.23	0.22	0.27	0.14	0.18	0.01	0.00	0.00	0.21	0.06	0.14	0.12

Peak no. [a]	Compound	Islay a [b]	Islay b [b]	Islay c [b]	Islay d [b]	Islay e [b]	Islay f [b]	Orkney a [b]	Orkney b [b]	St F a [b]	St F b [b]	St F f [b]	St F g [b]
36	4-acetylphenol	1.79	1.84	1.88	1.48	1.31	0.42	0.37	0.26	1.87	0.68	1.45	1.11
37	2-Chlorophenol	0.77	0.71	0.76	0.50	0.38	0.11	0.00	0.00	0.40	0.11	0.36	0.20
38	Unknown (123,152)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	Guaiacol	35.62	27.48	31.95	24.65	18.82	5.46	1.12	0.57	26.43	8.27	21.12	16.10
40	2-methylnaphthalene	0.87	0.85	0.77	0.39	0.40	0.17	0.03	0.00	0.41	0.15	0.44	0.67
41	Unknown (123,138)	0.28	0.27	0.37	0.29	0.22	0.00	0.00	0.00	0.52	0.16	0.20	0.27
42	2,6-dimethylphenol	2.56	2.53	2.86	1.76	2.35	0.25	0.09	0.07	2.63	0.87	2.18	1.80
43	1-methylnaphthalene	0.79	0.75	0.64	0.33	0.31	0.17	0.00	0.01	0.35	0.14	0.36	0.53
44	Hydroxymethylacetophenone	0.42	0.41	0.44	0.26	0.28	0.06	0.00	0.00	0.46	0.15	0.32	0.29
45	Benzylnitrile	0.77	0.79	0.90	0.47	0.70	0.00	0.00	0.00	0.93	0.28	0.93	1.05
46	Unknown (135, 150)	0.24	0.22	0.23	0.13	0.15	0.02	0.00	0.00	0.27	0.10	0.18	0.19
47	Methoxymethylphenol	1.01	0.76	1.02	0.86	0.53	0.06	0.00	0.00	1.11	0.33	0.62	0.62
48	Methylguaiacol	11.26	8.88	11.57	8.74	6.66	1.49	0.33	0.18	13.65	4.78	8.01	8.23
49	Quinoline	1.65	1.50	1.11	0.37	1.80	0.36	0.12	0.00	0.40	0.18	0.91	0.85
50	Dimethoxytoluene 2	0.00	0.06	0.09	0.00	0.02	0.00	0.00	0.00	0.19	0.00	0.04	0.03
51	*o*-Cresol	29.75	23.72	26.28	18.83	17.82	4.42	0.94	0.45	24.00	9.76	20.06	16.71
52	Phenol	50.18	36.59	43.45	33.24	32.19	9.42	1.89	1.11	43.00	18.45	31.53	30.01
53	Biphenyl	0.29	0.24	0.19	0.09	0.10	0.07	0.00	0.00	0.11	0.02	0.14	0.23
54	Ethylguaiacol	20.05	15.14	21.84	14.62	12.08	2.24	0.58	0.35	20.52	7.16	12.91	11.74

Peak no. [a]	Compound	Islay a [b]	Islay b [b]	Islay c [b]	Islay d [b]	Islay e [b]	Islay f [b]	Orkney a [b]	Orkney b [b]	St F a [b]	St F b [b]	St F f [b]	St F g [b]
55	Indanone	0.86	0.74	0.70	0.46	0.58	0.23	0.09	0.00	0.70	0.43	0.63	0.74
56	Methylindanone 2	0.76	0.78	0.72	0.34	0.59	0.00	0.00	0.00	0.75	0.36	0.63	0.53
57	2-Ethylphenol	12.04	9.59	11.77	7.85	6.46	1.45	0.31	0.12	7.73	3.00	7.09	4.79
58	Dimethylphenol 2 + 3	17.39	14.02	15.75	10.44	11.43	2.05	0.46	0.20	13.70	5.66	11.30	9.33
59	*p*-Cresol	19.87	14.51	16.90	12.39	14.95	2.90	0.55	0.25	14.40	6.10	11.41	11.51
60	*m*-Cresol	9.29	6.56	7.79	5.79	6.05	1.56	0.29	0.11	8.59	3.72	6.25	6.28
61	Propylguaiacol	1.69	1.27	1.69	0.98	0.91	0.12	0.02	0.00	1.17	0.37	0.80	0.59
62	C3 phenol 1	3.46	2.67	3.63	2.18	1.92	0.31	0.00	0.00	1.99	0.79	1.73	1.12
63	C3 phenol 2	1.10	0.82	1.12	0.68	0.58	0.11	0.00	0.00	0.69	0.26	0.57	0.38
64	Dimethylphenol 4	3.53	2.60	2.89	1.85	2.28	0.37	0.00	0.00	2.70	1.18	2.06	1.87
65	C3 phenol 3	8.66	6.86	9.02	4.95	5.69	0.80	0.17	0.00	5.94	2.53	5.02	3.74
66	C3 phenol 4	2.19	1.67	1.94	1.23	1.34	0.14	0.04	0.00	1.53	0.63	1.24	0.88
67	4-Ethylphenol	45.13	29.91	36.16	24.59	32.21	6.28	1.72	1.09	27.54	12.49	22.70	20.76
68	Eugenol	0.42	0.31	0.44	0.23	0.31	0.00	0.00	0.00	0.36	0.17	0.21	0.21
69	3-Ethylphenol	4.58	3.13	3.75	2.45	3.03	0.59	0.15	0.07	3.20	1.54	2.62	2.52
70	Vinylguaiacol	0.60	0.48	0.80	0.52	0.37	0.07	0.10	0.00	0.41	0.60	0.37	0.19
71	C3 phenol 5	1.22	0.88	1.08	0.64	0.78	0.00	0.00	0.00	1.03	0.44	0.75	0.73
72	Dibenzofuran	0.85	0.77	0.63	0.28	0.34	0.21	0.00	0.00	0.36	0.15	0.48	0.70

[a] Compounds detected in industrial new-make spirit using GC-MS. Colour coding indicates class of compound: carbohydrate derivatives, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics and unknowns.

[b] Data are response ratios x 10^2 and are the average of two injections.

Appendix K

Table 3.23. Peat-derived compounds, and their aromas, detected in lab-scale peated new-make spirits using GC-O/MS.

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
1	?	14.80	1184			Sulphury, burnt			0	1	1	0	1	0	1
2	1,1-Diethoxyhexane	15.25	1201		174	Solvent		Fresh ethereal, somewhat herbaceous, slightly fruity & rum-like	1	0	1	2	1	2	10
3	3-Methylcyclopentanone	15.75	1220		98	Solvent, stale	Wood resin	Not listed	1	2	1	2	1	1	10
4	?	16.33	1242				Earthy, vegetably, dirty		0	1	1	0	0	1	1
5	?	16.42	1246			Earthy, stale	Earthy, woody, solvent		2	2	1	1	3	3	1
6	Ethyl *trans*-2-pentenoate	16.67	1255		128	Earthy, stale		Green, fruity, slightly fermented	1	2	1	1	1	1	100
7	Styrene	16.88	1263	1261	104	Sweet, solvent		Penetrating, gasoil, plastic-like, ethereal	1	1	0	2	1	1	1

Aroma no.[a]	Compound[b]	RT[c]	Estimated RI[d]	Literature RI[e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis)[f]	Gar[g]	Cas[g]	St F[g]	Gle[g]	Tom[g]	Ork[g]	Dilution value[h]
8	Ethyl 3-hexenoate	17.03	1269		142	Sweet, solvent		Fruity, green, sweet pineapple character	0	2	1	0	2	2	1
9	?	18.33	1318			Sweet, solvent			2	2	2	2	2	2	1
10	?	18.43	1322			Earthy, stale			0	0	0	1	1	0	1
11	Ethyl heptanoate	18.62	1329	**1352**	158	Sickly sweet, estery, plastic, solvent		Sweet, fruity, fermenty, rum, brandy, apple-peel & berry notes, waxy with a green winey nuance	2	2	2	2	2	2	1
12	?	18.83	1337			Burnt, stale, sulphurs			0	0	1	1	1	1	1
13	?	19.15	1349				Earthy, stale, meaty, sweaty, sour		2	2	2	2	2	2	1
14	3-Ethylcyclopentanone	19.37	1358		112	Earthy, solvent, burnt			1	1	2	1	1	2	10
15	Unknown (95,140)	19.60	1366			Solvent, burnt			2	2	2	2	2	2	100
16	Ethyl 5-heptenoate	20.60	1404		156	Sweet, solvent	Plasticy, oily, pickled onions, vinegar		2	3	1	2	2	2	100

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
17	?	21.25	1429			Earthy, solvent, burnt			2	1	1	2	2	2	10
18	?	22.08	1460			Earthy, stale			1	0	0	1	1	1	10
19	Unknown (67,110)	22.63	1481			Earthy, stale			1	1	1	0	0	2	100
20	Ethyl 7-octenoate	23.12	1500	1489	170	Plastic, sickly sweet			1	1	0	1	2	1	10
21	?	23.32	1508			Earthy, stale			2	2	1	2	2	2	10
22	6-Hepten-1-ol	23.77	1525		114		Green, musty, meaty, cheesy, sour feet		1	2	0	1	0	0	1
23a	Trimethylcyclopentenone	24.12	1538		124	Earthy, stale		Sweet aromatic, ethereal, musty, caramellic, somewhat bitter almond-like (2-acetylfuran)	2	0	0	2	0	0	1
23b	Acetylfuran			1487	110										
24	2,3-Dimethyl-2-cyclopenten-1-one	25.33	1584		110	Earthy, stale		Fresh, burnt, somewhat caramellic, fruity & roasted	1	0	1	1	1	1	10
25	Ethyl 8-nonenoate	25.85	1604		184	Sweet, plasticy			0	1	0	0	0	1	1

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
26	?	25.97	1608			Estery, fresh, herbal, sickly sweet, minty, boiled potatoes, waxy	Plasticy, sour, gluey, solvent, dirty, oily		1	3	2	2	2	3	1
27	?	26.12	1614			Herbal, plasticy, sweet			1	0	0	0	0	1	1
28	?	26.55	1630			Plasticy, solventy			1	0	0	1	1	1	1
29	?	26.75	1638			Plasticy			2	1	1	1	0	0	1
30	?	26.93	1645			Earthy, stale	Sweaty, earthy		2	2	1	1	2	3	1
31	Acetylmethylfuran	27.33	1660	1848	124	Earthy, stale, green	Elderberry leaves, green wood sap	Nutty, cocoa-like with a toasted bready nuance (2-acetyl-5-methylfuran)	4	4	4	2	3	4	100
32	?	27.63	1671			Plasticy, herbal, sweet, earthy, minty			1	2	2	2	2	2	1
33	?	27.78	1677			Earthy, stale	Earthy, rotten vegetables		1	3	1	3	2	0	10

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
34	Methylfuranylpropanone	28.97	1722		138	Stale, solvent, earthy	Plasticy, solventy		2	2	2	3	4	1	100
35	?	29.13	1728				Solventy, sweaty, earthy		0	1	2	0	0	0	1
36a **36b** **36c**	Dimethoxybenzene Pentanoic acid Ethyl undecanoate	29.73	1751		138 102 214	Burnt, phenolic	Plasticy, sour, waxy, oily, solventy	Aromatic, somewhat phenolic/medicinal, slightly spicy (1,2-dimethoxybenzene). Strongly acidic, caprylic, dairy-like with milky & cheesy nuances (pentanoic acid). Waxy, creamy, slight fruity with a coconut and cheesy nuance (ethyl undecanoate).	2	2	2	3	3	1	1
37	?	30.08	1764			Estery			0	0	0	2	1	1	10
38	1-Phenyl-2-propanone	30.20	1769		134	Sweet, stale, earthy		Aromatic, spicy with fruity note	1	1	1	2	2	1	1
39	?	30.60	1784			Earthy, stale			1	2	2	2	0	1	100

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
40	Methylacetophenone 1	30.97	1798		134	Estery, phenols, sweaty, cheesy, burnt plastic	Fruity, heavy, nearly phenolic	Sweet aromatic, cumin-like, vanilla, cream, fruity, cherry, bitter almond, hay-like	2	1	1	0	2	0	100
41	?	31.25	1809				Dirty, sour, phenolic		1	0	0	1	1	0	1
42	?	31.40	1814			Stale	Phenolic, spicy		0	1	1	1	3	2	1
43	Methylacetophenone 2	31.67	1824		134	Estery, stale, phenolic	Phenolic, woody, disinfectant, plasticy	Sweet aromatic, cumin-like, vanilla, cream, fruity, cherry, bitter almond, hay-like	1	3	1	1	2	1	100
44	Dimethoxytoluene 1	31.78	1829		152	Phenolic, burnt		Sweet aromatic, somewhat phenolic, medicinal, spicy	2	2	1	1	1	1	1
45	2-Chlorophenol	32.80	1867		128	Phenolic, medicinal, TCP, plasters	Phenolic, earthy, meat, sour, rotten, medicinal		2	3	2	4	2	2	10
46	Dimethoxytoluene 2	32.97	1874		152	Smoky, phenolic, burnt		Sweet aromatic, somewhat phenolic, medicinal, spicy	2	2	2	2	2	2	10

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
47	Guaiacol	33.20	1883	**1868**	124	Smoky, phenolic to stale, earthy	Phenolic, medicinal, wood, curry, spicy	Aromatic, phenolic, burnt; woody, bacon, savoury, smoky and medicinal	4	4	4	4	3	4	1000
48	Unknown (123,138)	33.48	1893			Sweet, perfumy, medicinal , spicy	Spicy, phenolic, sweet		3	3	3	2	1	2	1
49	Dimethylphenol 1	34.23	1922	1889	122	Phenolic/ plasters		Aromatic, phenolic, slightly spicy, musty, chemical, stringent	2	1	2	2	2	2	1000
50	Hydroxymethylacetophenone	34.97	1950		150	Plasters, phenols	Chlorinated swimming pools		4	4	4	3	4	4	10
51	Methoxymethylphenol	35.13	1956		138	Phenolic, smoky, sweet	Phenolic, spicy, clove, eugenol	Dusty, reminiscent of oak- and treemoss (3-methoxy-5-methylphenol)	3	4	4	4	2	4	1000
52	Methylguaiacol	35.48	1969	**1968**	138	Sweet, vanilla		Spicy, medicinal, vanilla, clove-like, phenolic nuances	2	2	2	2	2	2	10

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
53	Quinoline	35.92	1986		129	Stale, phenols, rubbery, barn	Vegetably, sour, earthy	Putrid, narcotic, on dilution somewhat earthy green	2	0	1	2	2	1	100
54	*o*-Cresol	36.18	1996	**2026**	108	Phenolic, medicinal		Phenolic	2	2	2	2	2	2	100
55	?	36.70	2015			Plasters, synthetic			0	2	1	1	1	1	10
56	Ethylguaiacol	37.10	2031	**2034**	152	Sweet, spicy, phenolic	Phenolic, spicy, sweet, cloves	Spicy, smoke-like, medicinal, woody and sweet vanilla nuances, on dilution bacon-like	3	3	3	3	2	4	100
57	Unknown (137,152)	37.23	2035			Sweet, spicy, phenolic, plasters, burnt			2	2	2	2	2	2	100
58	2-Ethylphenol	37.63	2051		122	Estery, crayons to phenols, plasters	Sour, chlorinated swimming pools	Aromatic, phenolic, medicinal	3	3	3	2	3	3	100
59	Dimethylphenol 2 + 3	37.85	2059		122	Medicinal		Aromatic, phenolic, slightly spicy, musty, chemical, stringent	2	2	1	0	2	2	100

Aroma no. [a]	Compound [b]	RT [c]	Estimated RI [d]	Literature RI [e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis) [f]	Gar [g]	Cas [g]	St F [g]	Gle [g]	Tom [g]	Ork [g]	Dilution value [h]
60	Dimethylphenol 2 + 3	37.92	2062		122	Medicinal	Disinfectant, manure	Aromatic, phenolic, slightly spicy, musty, chemical, stringent	1	0	1	1	1	1	100
61a **61b**	*p*-Cresol *m*-Cresol	38.17	2071	**2079** **2095**	108 108	Barnyard	Cowpats, manure	Powerful cresylic (*p*-cresol). Phenolic (*m*-cresol)	3	4	2	3	4	3	1000
62	?	38.42	2081				Vegetably, sour, manure		0	0	0	0	1	1	1
63	Propylguaiacol	38.87	2098	2138	166	Phenolic, spicy	Burning electrics, oily	Aromatic, spicy, clove-like	3	2	3	3	3	3	100
64	C3 phenol	39.20	2110		136	Medicinal, phenolic, burnt		Phenolic, aromatic, somewhat spicy (5-ethyl-*m*-cresol)	0	1	1	2	0	0	10
65a **65b**	4-Ethylphenol Eugenol	40.15 40.15	2146 2146	**2095** 2169	122 164	Barnyard, medicinal, spicy	Plasticy, earthy	Phenolic, aromatic, slightly spicy (4-Ethylphenol). Strongly warm spicy, clove-like (eugenol).	2	2	2	2	3	3	100
66	3-Ethylphenol	40.25	2150	2221	122	Burnt, barns, medicinal		Phenolic, aromatic, somewhat spicy	0	0	1	1	0	1	10

Aroma no.[a]		Compound[b]	RT[c]	Estimated RI[d]	Literature RI[e]	MW	Odour description (assessor a)	Odour description (assessor b)	Aroma (Bacis)[f]	Gar[g]	Cas[g]	St F[g]	Gle[g]	Tom[g]	Ork[g]	Dilution value[h]
67	?		40.83	2172			Burnt, stale			1	0	1	0	1	1	1
68	?		41.32	2191			Phenolic, burnt	Plasticy, earthy		1	3	1	3	2	1	10
69	?		41.88	2212			Plasters	Scorched electrics, plastic		0	0	2	1	1	1	100
70	?		42.25	2226			Burnt electrics			1	0	0	1	0	0	1
71	?		42.78	2246				Burning electrics, stale, oily		0	0	1	0	1	0	1
72	?		43.95	2291			Burnt			2	1	2	1	2	2	10
73	?		45.35	2344			Tarry, burnt			0	1	1	0	0	1	1
74	?		46.08	2371			Tarry, burnt			0	0	0	1	0	1	1
75	?		49.07	2485			Burnt			1	0	0	0	1	0	1

[a] Letters after aroma numbers indicate where more than one compound eluted at the same time as an aroma.

[b] Where quantifiable ions were present but no compound could be identified, the unknown compound was identified by its characteristic ions.

[c] Retention Time at which aroma was first detected.

[d] Retention Index at which aroma was first detected.

[e] Literature Retention Index data taken from [153,154,159-161,163]. Estimated RI were calculated as described in Chapter 2.4.2 using literature RI data for selected compounds (identified in bold as well as the ethyl esters listed in Chapter 3.3.9). Literature Retention Index data for the additional ethyl esters were taken from [154,163,164].

[f] Aromas were compared with those listed on the Bacis 98 database of flavour (raw) materials.

[g] The total number of times each aroma was detected in two runs by two assessors was reported for each sample.

[h] Dilution values show the highest dilution factor at which aromas were detected by assessor a (using Castlehill spirit).

Table 3.24. Response ratios for peat-derived aroma active compounds detected by GC-O/MS in lab-scale peated spirits and lab-scale unpeated spirit.

Aroma no.[a]	Compound	Estimated RI	QI	Cas[b]	Gar[b]	Gle[b]	Ork[b]	St F[b]	Tom[b]	Unpeat[b]
2	1,1-Diethoxyhexane	1201	103,129	0.72 (19.38)	0.99 (32.35)	0.95 (33.33)	1.21 (12.21)	0.47 (27.25)	0.87 (46.46)	0.18 (85.46)
3	3-Methylcyclopentanone	1220	98	7.47 (12.19)	7.12 (6.25)	7.11 (4.58)	6.78 (10.15)	6.83 (7.33)	8.93 (7.68)	0.00 (0.00)
6	Ethyl *trans*-2-pentenoate	1255	83,99	9.07 (6.64)	11.34 (5.72)	13.64 (4.82)	10.10 (7.89)	6.62 (15.02)	9.90 (4.94)	1.71 (18.35)
7	Styrene	1263	103,104	5.66 (8.68)	4.28 (3.91)	7.80 (3.57)	1.43 (6.56)	0.73 (8.03)	3.72 (3.08)	0.00 (0.00)
8	Ethyl 3-hexenoate	1269	69	2.03 (9.01)	2.12 (6.21)	2.21 (7.64)	2.18 (5.35)	1.68 (7.13)	2.13 (7.02)	0.00 (0.00)
11	Ethyl heptanoate	1329	88,113	14.44 (6.00)	17.08 (4.42)	16.38 (3.69)	21.97 (4.74)	11.58 (4.52)	11.19 (1.10)	0.85 (10.51)
14	3-Ethylcyclopentanone	1358	83,112	10.35 (9.58)	10.51 (5.19)	10.38 (4.40)	9.71 (7.28)	9.16 (6.49)	11.26 (6.49)	0.68 (16.14)
15	Unknown (95,140)	1366	95,140	83.16 (6.98)	88.05 (2.58)	96.13 (1.40)	144.45 (6.32)	87.66 (2.58)	196.68 (2.35)	0.64 (6.09)
16	Ethyl 5-heptenoate	1404	68,88	0.67 (4.97)	0.87 (10.32)	0.76 (5.08)	1.14 (8.76)	0.56 (5.43)	0.49 (5.59)	0.00 (0.00)
19	Unknown (67,110)	1481	67,110	0.58 (7.22)	0.66 (4.62)	0.60 (4.93)	0.69 (5.34)	0.55 (9.11)	0.54 (10.50)	0.06 (84.86)
20	Ethyl 7-octenoate	1500	55,88	4.18 (3.70)	5.47 (4.38)	5.11 (3.33)	7.86 (3.27)	3.05 (4.89)	3.36 (4.38)	0.00 (0.00)
22	6-Hepten-1-ol	1525	54,67	1.37 (6.70)	1.42 (3.20)	1.44 (5.27)	1.31 (4.99)	0.75 (4.15)	0.74 (4.40)	0.00 (0.00)
23a	Trimethylcyclopentenone	1538	81,109,124	2.52 (6.66)	2.65 (5.89)	2.81 (5.67)	2.57 (5.90)	2.12 (8.00)	2.78 (9.22)	0.00 (0.00)
23b	Acetylfuran	1538	95,110	7.62 (8.16)	8.25 (5.97)	9.04 (5.27)	9.23 (6.41)	6.81 (7.47)	16.10 (7.17)	0.00 (0.00)
24	2,3-Dimethyl-2-cyclopenten-1-one	1584	67,110	4.51 (6.90)	4.49 (5.74)	4.70 (5.42)	4.35 (5.41)	4.05 (6.43)	5.28 (6.18)	0.00 (0.00)
25	Ethyl 8-nonenoate	1604	88,138	2.11 (7.25)	2.66 (6.56)	2.53 (4.49)	3.35 (5.49)	1.80 (7.43)	1.91 (5.72)	0.33 (41.04)
31	Acetylmethylfuran	1660	109,124	3.52 (7.61)	4.04 (6.07)	4.45 (6.43)	4.28 (7.91)	3.13 (11.46)	6.75 (8.90)	1.31 (9.02)
34	Methylfuranylpropanone	1722	109,138	1.30 (2.97)	1.43 (3.53)	1.66 (4.50)	1.69 (9.83)	1.16 (2.01)	2.39 (7.27)	0.01 (282.84)
36a	Dimethoxybenzene	1751	123,138	0.89 (2.94)	0.78 (3.45)	0.95 (4.68)	0.37 (7.31)	1.36 (2.46)	0.36 (6.33)	0.00 (0.00)
36b	Pentanoic acid	1751	60,73	2.50 (13.90)	2.84 (5.73)	2.94 (14.22)	3.32 (11.21)	1.71 (15.06)	2.24 (8.38)	0.00 (0.00)

Aroma no.[a]	Compound	Estimated RI	QI	Cas[b]	Gar[b]	Gle[b]	Ork[b]	St F[b]	Tom[b]	Unpeat[b]
36c	Ethyl undecanoate	1751	88,101	1.17 (12.36)	1.49 (7.33)	1.22 (6.44)	1.80 (9.68)	1.34 (4.16)	1.16 (6.97)	0.76 (8.35)
38	1-Phenyl-2-propanone	1769	91,134	2.16 (3.23)	2.07 (3.69)	2.31 (5.06)	1.89 (1.59)	1.54 (2.55)	1.85 (3.36)	0.00 (0.00)
40	Methylacetophenone 1	1798	91,119,134	4.96 (2.75)	5.92 (3.71)	6.15 (3.50)	6.07 (4.08)	4.57 (2.26)	6.90 (3.56)	0.24 (107.92)
43	Methylacetophenone 2	1824	91,119,134	5.17 (4.25)	5.89 (4.27)	6.26 (2.97)	5.47 (4.43)	5.29 (4.79)	6.30 (4.41)	0.57 (138.31)
44	Dimethoxytoluene 1	1829	137,152	2.64 (1.93)	1.83 (3.27)	2.41 (3.99)	1.03 (3.95)	3.72 (1.55)	0.96 (5.05)	0.00 (0.00)
45	2-Chlorophenol	1867	128	1.80 (5.01)	1.72 (3.50)	2.55 (4.44)	1.48 (4.29)	0.82 (3.05)	3.14 (3.41)	0.00 (0.00)
46	Dimethoxytoluene 2	1874	123,152	0.64 (5.67)	0.51 (5.55)	0.67 (6.61)	0.32 (10.97)	0.94 (4.68)	0.37 (5.69)	0.00 (0.00)
47	Guaiacol	1883	109,124	163.69 (8.88)	136.01 (7.16)	167.95 (6.47)	77.63 (6.30)	127.40 (8.50)	82.64 (7.45)	0.36 (44.83)
48	Unknown (123,138)	1893	123,138	2.72 (5.15)	1.90 (6.80)	3.23 (10.60)	0.74 (13.13)	3.09 (9.24)	0.99 (17.74)	0.00 (0.00)
49	Dimethylphenol 1	1922	107,122	10.90 (4.46)	10.06 (4.40)	11.81 (4.24)	7.03 (3.81)	9.16 (2.80)	8.39 (4.35)	0.00 (0.00)
50	Hydroxymethylacetophenone	1950	135,150	1.93 (3.08)	2.12 (4.23)	2.40 (3.71)	1.91 (5.19)	1.51 (2.55)	2.47 (3.57)	0.00 (0.00)
51	Methoxymethylphenol	1956	123,138	4.12 (3.89)	2.92 (4.70)	4.22 (8.08)	1.42 (8.38)	4.49 (4.97)	1.66 (12.45)	0.00 (0.00)
52	Methylguaiacol	1969	123,138	87.21 (3.71)	63.30 (3.96)	93.89 (7.46)	35.66 (5.90)	86.40 (4.11)	47.47 (10.90)	0.19 (98.44)
53	Quinoline	1986	129	0.61 (8.77)	0.67 (2.77)	0.64 (8.21)	0.55 (11.27)	0.56 (8.15)	0.52 (9.00)	0.00 (0.00)
54	*o*-Cresol	1996	107,108	121.23 (5.20)	112.25 (3.97)	125.82 (4.83)	81.65 (2.66)	105.93 (2.82)	109.66 (4.91)	0.22 (78.82)
56	Ethylguaiacol	2031	137,152	82.72 (3.28)	58.79 (3.14)	85.19 (7.55)	35.60 (5.90)	67.26 (2.10)	42.82 (8.52)	0.22 (97.38)
57	Unknown (137,152)	2035	137,152	1.15 (6.74)	0.81 (7.58)	0.96 (8.19)	0.34 (14.05)	1.08 (9.08)	0.32 (16.07)	0.00 (0.00)
58	2-Ethylphenol	2051	107,122	21.48 (4.42)	19.10 (3.59)	21.17 (4.51)	14.05 (2.09)	15.33 (2.52)	13.48 (3.16)	0.00 (0.00)
59/60	Dimethylphenol 2 + 3	2059/2062	107,122	66.17 (3.66)	56.28 (3.69)	65.62 (4.75)	40.14 (1.90)	58.61 (2.28)	53.95 (4.34)	0.10 (113.45)
61a	*p*-Cresol	2071	108	53.43 (3.17)	45.00 (4.77)	57.09 (5.71)	28.69 (4.27)	42.22 (3.53)	44.40 (7.31)	0.02 (282.84)
61b	*m*-Cresol	2071	108	22.00 (3.08)	19.43 (3.96)	18.68 (5.72)	14.34 (2.76)	22.91 (2.88)	21.86 (8.32)	0.02 (245.37)
63	Propylguaiacol	2098	137,166	5.77 (4.28)	3.79 (2.08)	6.03 (7.95)	1.97 (6.69)	6.54 (1.93)	2.42 (7.06)	0.00 (0.00)
64	C3 phenol	2110	121,136	6.32 (3.04)	5.05 (4.18)	5.97 (6.02)	3.33 (1.72)	4.30 (4.07)	3.29 (3.01)	0.00 (0.00)
65a	4-Ethylphenol	2146	107,122	95.13 (3.15)	76.37 (3.83)	92.57 (6.43)	52.65 (2.16)	73.47 (2.97)	71.82 (6.71)	1.00 (17.60)
65b	Eugenol	2146	149,164	1.94 (3.79)	1.15 (2.16)	1.67 (8.20)	0.65 (5.91)	2.42 (2.10)	0.78 (7.76)	0.00 (0.00)

Aroma no.[a]	Compound	Estimated RI	QI	Cas[b]	Gar[b]	Gle[b]	Ork[b]	St F[b]	Tom[b]	Unpeat[b]
66	3-Ethylphenol	2150	107,122	12.69 (3.85)	10.40 (3.85)	9.67 (6.76)	6.81 (4.17)	11.43 (3.70)	8.78 (7.21)	0.01 (282.84)

[a] Aroma numbers refer to those in Table 3.23. Colour coding indicates class of compound: carbohydrate derivatives, guaiacols, syringols, phenols, nitrogen-containing compounds, aromatics and other compounds.

[b] Data are response ratios x 10^2 and are the means of three new-make spirit samples (data for each sample was recorded as the mean of two injections) . Data in brackets are % RSDs.

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Table 3.25. Comparison of aromas detected in lab-scale and industrial peated new-make spirits using GC-O/MS.

Aroma no.[a]	Dilution value[b]	Aroma detected in industrial samples	Compound[c]	Peak area in lab spirit[d]	Peak area in industrial spirit[d]	Industrial: lab-scale spirit (%)[e]
1	1	Yes	?			
2	10	Yes	1,1-Diethoxyhexane	0.87 (39.42)	1.44 (42.77)	166.63
3	10	Yes	3-Methylcyclopentanone	7.37 (12.81)	1.07 (8.56)	14.54
4	1		?			
5	1	Yes	?			
6	100	Yes	Ethyl *trans*-2-pentenoate	10.11 (22.34)	1.33 (22.31)	13.18
7	1		Styrene	3.94 (61.91)	0.30 (14.63)	7.69
8	1		Ethyl 3-hexenoate	2.06 (11.08)	0.22 (25.37)	10.66
9	1	Yes	?			
10	1		?			
11	1		Ethyl heptanoate	15.44 (24.31)	1.75 (46.88)	11.31
12	1		?			
13	1		?			
14	10		3-Ethylcyclopentanone	10.23 (9.11)	1.47 (6.56)	14.35
15	100	Yes	Unknown (95,140)	116.02 (36.45)	8.23 (35.06)	7.09
16	100	Yes	Ethyl 5-heptenoate	0.75 (30.40)	0.00 (0.00)	0.00
17	10	Yes	?			
18	10	Yes	?			
19	100		Unknown (67,110)	0.60 (11.52)	0.17 (18.72)	27.64
20	10		Ethyl 7-octenoate	4.84 (33.73)	0.62 (18.84)	12.85
21	10	Yes	?			
22	1		6-Hepten-1-ol	1.17 (26.68)	0.31 (14.01)	26.75
23	1	Yes	Trimethylcyclopentenone	2.57 (11.10)	0.69 (11.33)	26.84
23	1	Yes	Acetylfuran	9.51 (33.23)	2.12 (11.31)	22.29
24	10	Yes	2,3-Dimethyl-2-cyclopenten-1-one	4.56 (10.10)	1.44 (1.14)	31.48
25	1		Ethyl 8-nonenoate	2.39 (23.00)	0.31 (11.47)	12.85
26	1	Yes	?			
27	1		?			
28	1	Yes	?			
29	1	Yes	?			
30	1		?			
31	100	Yes	Acetylmethylfuran	4.36 (28.03)	1.30 (14.72)	29.89

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Aroma no.[a]	Dilution value[b]	Aroma detected in industrial samples	Compound[c]	Peak area in lab spirit[d]	Peak area in industrial spirit[d]	Industrial: lab-scale spirit (%)[e]
32	1	Yes	?			
33	10	Yes	?			
34	100	Yes	Methylfuranylpropanone	1.60 (25.90)	0.49 (40.71)	30.68
35	1		?			
36	1	Yes	Dimethyoxybenzene	0.79 (44.82)	0.27 (26.68)	34.42
36	1	Yes	Pentanoic acid	2.59 (23.10)	1.05 (38.06)	40.52
36	1	Yes	Ethyl undecanoate	1.36 (18.62)	1.76 (19.78)	129.38
37	10	Yes	?			
38	1	Yes	1-Phenyl-2-propanone	1.97 (13.14)	0.10 (115.47)	5.06
39	100	Yes	?			
40	100	Yes	Methylacetophenone 1	5.76 (14.03)	1.97 (32.87)	34.26
41	1		?			
42	1		?			
43	100	Yes	Methylacetophenone 2	5.73 (8.87)	1.81 (26.72)	31.62
44	1		Dimethoxytoluene 1	2.10 (46.26)	0.64 (2.03)	30.32
45	10	Yes	2-Chlorophenol	1.92 (39.63)	1.10 (5.59)	57.54
46	10	Yes	Dimethoxytoluene 2	0.58 (36.77)	0.11 (11.43)	19.85
47	1000	Yes	Guaiacol	125.89 (29.37)	49.05 (9.35)	38.96
48	1	Yes	Unknown (123,138)	2.11 (47.99)	1.12 (40.53)	52.99
49	1000	Yes	Dimethylphenol 1	9.56 (17.19)	8.28 (5.15)	86.67
50	10	Yes	Hydroxymethylacetophenone	2.05 (16.32)	0.97 (20.64)	46.99
51	1000	Yes	Methoxymethylphenol	3.14 (40.29)	2.38 (36.04)	75.90
52	10	Yes	Methylguaiacol	68.99 (32.52)	27.24 (38.09)	39.48
53	100	Yes	Quinoline	0.59 (11.80)	2.61 (71.85)	441.51
54	100	Yes	*o*-Cresol	109.42 (13.71)	113.89 (13.96)	104.08
55	10		?			
56	100	Yes	Ethylguaiacol	62.06 (30.69)	39.48 (29.38)	63.61
57	100	Yes	Unknown (137,152)	0.78 (44.41)	0.88 (27.04)	113.20
58	100	Yes	2-Ethylphenol	17.44 (19.37)	20.76 (8.03)	119.03
59	100	Yes	Dimethylphenol 2 + 3			
60	100	Yes	Dimethylphenol 2 + 3	56.80 (15.90)	74.72 (8.33)	131.55
61	1000	Yes	*p*-Cresol	45.14 (20.83)	37.35 (4.37)	82.76
61	1000	Yes	*m*-Cresol	19.87 (15.45)	19.85 (16.72)	99.87
62	1		?			
63	100	Yes	Propylguaiacol	4.42 (41.33)	2.96 (11.25)	66.99
64	10	Yes	C3 phenol	4.71 (25.73)	5.77 (2.67)	122.44
65	100	Yes	4-Ethylphenol	77.00 (19.14)	82.97 (9.02)	107.75
65	100	Yes	Eugenol	1.44 (45.01)	0.82 (8.91)	57.47

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Aroma no.[a]	Dilution value[b]	Aroma detected in industrial samples	Compound[c]	Peak area in lab spirit[d]	Peak area in industrial spirit[d]	Industrial: lab-scale spirit (%)[e]
66	10		3-Ethylphenol	9.96 (19.65)	11.54 (2.24)	115.85
67	1	Yes	?			
68	10	Yes	?			
69	100		?			
70	1	Yes	?			
71	1		?			
72	10	Yes	?			
73	1	Yes	?			
74	1	Yes	?			
75	1		?			

[a] Aroma numbers refer to those listed in Table 3.23.

[b] Dilution values are as described in Table 3.23.

[c] Where more than one compound eluted at the time corresponding to an aroma, the compounds are highlighted yellow. Where data suggests that the identity of a compound responsible for an aroma may be incorrect, the compound is highlighted red.

[d] Data are response ratios x 10^2. For lab-scale spirit these data are the means of the values obtained for the six lab-scale peated spirits and for the industrial spirit these are the means of the values obtained from two highly peated spirits. Data in brackets are % RSDs.

[e] The mean response ratio of the industrial samples is reported as a percentage of the mean lab-scale peak response ratio.

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